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CONTENTS

THE DIFFERENTIAL EFFECT OF ENVIRONMENTAL FACTORS UPON *MICROBRACON HEBETOR* SAY (HYMENOPTERA: BRACONIDAE) AND ITS HOST, *EPHESTIA KUHNIELLA* ZELLER (LEPIDOPTERA: PYRALIDAE). II.

NELLIE M. PAYNE

THE ROLE OF COPPER IN THE SETTING, METAMORPHOSIS, AND DISTRIBUTION OF THE AMERICAN OYSTER, *OSTREA VIRGINICA.*

HERBERT F. PRYTHERCH

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THE DIFFERENTIAL EFFECT OF ENVIRONMENTAL FACTORS UPON *MICROBRACON HEBETOR* SAY (HYMENOPTERA: BRACONIDAE) AND ITS HOST, *EPHESTIA KÜHNIELLA* ZELLER (LEPIDOPTERA: PYRALIDAE). II

I. INTRODUCTION

The Problem of Host-Parasite Balance

In spite of the enormous reproductive capacities of insects, they do not cover the earth completely. Rather, a given population tends to saturate its immediate environment and then come to an equilibrium. The point of equilibrium may not be absolutely fixed. If the environment changes very slightly, a former equilibrium may no longer hold. Such a change in environment may be either physical, as temperature, light, or humidity, or it may be biological, as the introduction of a new parasite, a predator, or a disease. Until comparatively recently there was no mathematical formula worked out to take care of even a simple problem in determination of population equilibria. However, by the use of life tables and census tabulations, Pearl (1925) was able to find a characteristic logistic curve which describes human population. For each nation in which vital statistics have been kept there appears to be the same type of curve; and if one holds that the same type of curve describes a similar process, one may believe that a similar if not identical biological event is taking place in each of the countries of western Europe with regard to increase in population. Pearl and Parker (1922) showed that Drosophila reared in an environment of fixed size tended to saturate the environment or to reach a constant, after which the population tended to be stationary. Chapman (1928) showed that *Tribolium confusum* Duval also tended to saturate its environment and then to come to a fixed population. Somewhat earlier (1925) he had formulated his hypothesis, which may be expressed mathematically by saying that $C = Bp/R$ or that concentration of insects is equal to the biotic potential divided by the environmental resistance. The biotic potential is the potential increase of the numbers of an insect. This potential is the increase if there were no hindrances such as disease, parasites, or unfavorable weather. Blunk (1929) has used another formula to express this central idea of a population and environment equilibrium. Chapman has measured the potential and the concentration. Blunk and Bremer have measured the "vernichtung's coefficient" or "coefficient of destruction," which may be expressed by the formula $q = 1 - \frac{bc}{ac}$ where q is the coefficient of destruction, a, the offspring per female, b, the

ratio of females to the total population, and c, the number of generations in a year. This destruction coefficient is expressed as the percentage of the progeny of one female which must die before maturity, if the population of the insect is to be kept constant. To give some idea of how great the coefficient of destruction must be, one of the insects used in this study may serve as an illustration, namely *Ephestia kühniella* Zeller, with a total period from egg-laying to egg-laying of 62 days at 32°C; with 52.5% of the adults females, and the number of eggs laid per female 181. The offspring from one female at the end of a year would be 30,428,590,122 counting five generations to a year. The percentage that must die to keep the number constant is 99.99999999993.

For *Microbracon hebetor*, the parasite of the Mediterranean flour moth, the biotic potential is even more striking. This insect has a short life cycle of 9 days from egg to egg at 32°C; the average number of eggs per female is about 111. On account of the peculiar manner of sex determination in this insect, whereby the unfertilized females produce males and the fertilized, females, a sex ratio in the two insects does not have the same effect on the biotic potential. In Microbracon if there is a disproportion of females in one generation, it will tend to be followed by a marked disproportion of males in the next. An additional complication is that occasionally there are biparental males which are usually sterile. For the purpose of calculation it will be assumed that half of the offspring are males and half females. The number of Microbracon at the end of a year would be $112.5^{40.55}$ or 14.8×10^{82} if no allowance were made for the overlapping of generations.

In this case the parasite, Microbracon, has a higher biotic potential and a shorter life cycle than the host, Ephestia. The problem is to find how long it will take Microbracon to overcome the host, and if the theoretical calculations do not agree with the results found experimentally, to find what factors enter which are not taken into account by a simple mathematical relation between host and parasite. One physical factor enters which may at times favor the host and at other times the parasite.

II. DISCUSSION OF THE LITERATURE

A. Literature on Host-Parasite Balance

1. *Host-Parasite Synchrony*

Thompson (1922) proposed a formula, later corrected by Chapman (1925), which could be used to calculate the number of generations required to exterminate a host. A simple case in which the parasite was assumed to lay only one egg on each host attacked was chosen. The environmental factors were also assumed to act equally favorably or unfavorably on both host and parasite.

The mathematical treatment of this simplified host-parasite relation may be stated as follows:

If n = the original population of the host.
l = the sex ratio of the host.
h = the number of young produced by each female host.
then $lh = w$ or the reproductive power of the host (reproductive potential).
Let p = the original population of the parasite.
f = the sex ratio of the parasite.
s = the number of young produced by each female parasite.
then $fs = z$, the reproductive power of the parasite (reproductive potential).
Thus nw = the number of hosts after the first generation.
pz = the number of parasites after the first generation.
$nw\text{-}pz$ = the number of surviving hosts after the first generation.
$(nw\text{-}pz)\ (w)$ = the number of hosts after the second generation.

The number of generations required for the number of parasites to equal the number of hosts is: $(nw^k. \ldots\ldots\ldots)\text{-}pz^k$

k may be found by using the mathematical transformation:

$$\Theta = \frac{w}{z} \qquad \beta = \frac{zp}{n(z\text{-}w)\text{-}zp} \qquad k = \frac{Log\ \Theta}{Log\ \beta}$$

If w is greater than z, or in other words if the reproductive power of the host is greater than that of the parasite, p can never equal h, and there is no theoretical possibility that the parasite can exterminate the host.

If $w = z$, or in other words, the reproductive powers of the host and parasite are equal, then $k = \frac{h}{p}$, or the number of generations needed by the parasite to equal the numbers of the host is equal to the quotient of the original number of the host, divided by the original number of the parasite.

The assumption that external environment acts alike or to the same degree on both host and parasite cannot be made safely. Differential action of some environmental factor on host and parasite is one of the cardinal principles in medicine, biology, and agriculture. One has only to think of the action of drugs on intestinal parasites, where it is essential that the parasite be killed, but the host uninjured; or of the action of fungicides and insecticides where it is the object again to destroy the parasite without injuring the host unduly, to realize how very generally there is some differential action of an agent on host and parasite.

Several well-known cases of differential action of weather on insects and their diseases and parasites illustrate the point. The chinch bug, *Blissus leucopterus* Say, is favored by hot, dry weather, and its parasite, the muscardine fungus, by wet weather. Another case, not perhaps so well-known,

was found by the author for *Eurymus philodice* God., and *Eurymus eurytheme* Boisd., two alfalfa caterpillars, and the polyhedral disease. In the early summer of 1918 about 2% of the caterpillars had an acute form of the disease and an additional 5% carried polyhedral bodies in their blood and might have been considered to have latent polyhedral disease. Cold rainy mornings followed by sunny afternoons transformed the latent disease into the acute form and in addition favored the spread of the disease to such an extent that all field work on the caterpillars had to be given up, since too few were to be found.

Escherich and Miyajima (1911, 1912) described an interesting relation between the polyhedral disease in the nun moth, *Porthetria monacha* Linn., and weather changes. By pricking the abdominal pro-legs of larvae with a sterile needle, these workers were able to obtain blood samples containing polyhedral disease from apparently healthy caterpillars. They wished to ascertain whether disease outbreaks were due to a sudden spread of polyhedral disease or to the development of disease already present in the bodies of caterpillars. They found that the latent or chronic form of the disease developed into an acute, fatal form when a sudden change in physical environment occurred. Exposure to bright sunlight or to sudden decrease in temperature resulted in transformation of a chronic to a fatal disease. Correlated field studies showed that sudden changes of weather were associated with polyhedral disease epidemics. It is of especial interest theoretically that not only was there an increase in number of cases of polyhedral disease, but also that there was actual augmentation of the number of polyhedral bodies found in individuals.

In discussing the differential action of heat on hosts and their parasites the two factors of thermal energy, namely, intensity and quantity, will be considered separately. Temperature is used to indicate the intensity factor of heat; calories represent the quantity factor of heat. It was found by the author (Payne 1927) that in cold-hardiness the two factors could be differentiated. Since it is seldom possible to express the quantity factor of heat in calories in developmental studies on insects, the time over which a given temperature acts is used to represent the quantity factor of heat.

2. *Differential action of the Intensity Factor of Heat on Host and Parasite*

Welter (1895) found that the eggs of *Bombyx mori* Linn. were very cold-hardy, being able to withstand -40°C, but their internal parasites (species not given) were killed by this temperature. Kalendar (1872) found that ichneumonid parasites of *Acronycta aceris* Linn. were killed by bringing the pupae which were parasitized into a warm room. In this case it is not quite clear whether the intensity factor or the capacity factor is involved. It is probable that both are concerned.

Kaufmann (1925) found that parasitized beetles of *Psylliodes chrysocephala* Linn. were more cold-hardy than non-parasitized.

3. *Differential action of the quantity factor of heat on host and parasite*

Webster and Phillips (1912) found that *Toxoptera graminum* Rond. was active and able to oviposit slightly below 40°F (4.4°C). Its chief parasite, *Aphidius testaceipes* Cress., was not active below 56°F (13.3°C). In Texas the generations overlap to some extent. The average number of young per viviparous female during the season from March to November was 28.2. The real number was probably larger, since some adults disappeared from the cages used in the experiment after producing from 1-10 young. Wingless females produced more offspring than winged. Webster and Phillips gave data on the fecundity of the viviparous female. At the end of 300 days the offspring from one such individual would equal 210^{15}. It is no wonder that an insect with such an enormous biotic potential as this is able to produce outbreaks.

Opposed to the high biotic potential of *Toxoptera graminum* Rond. are certain factors of environmental resistance including parasites. Of these, *Aphidius testaceipes* Cress., was the most important. Other parasites included *Aphidius confusus* Ashm., *Aphidius avenapis* Fitch, *Aphelinus mali* Hald., *Aphelinus nigritus* How. and *Aphelinus semiflavus* How. However, since the parasite, *Aphidus testaceipes*, is reported to winter in the body of the host, it is probably somewhat cold-hardy to the quantity factor of low temperature.

The average number of offspring per female was at least 94.6, since one female Aphidius could parasitize at least 94.6 Toxoptera. This average was based on 16 experiments. In cage experiments the female laid from 4 to 450 eggs. In some cases viviparous Toxoptera females produced a few young after they had been parasitized. Webster and Phillips observed that warm weather was more favorable to the parasites of Toxoptera than to the green bug itself. Rainfall, especially in the form of a cold drizzle, prevented the parasites from flying about and depositing their eggs. The problem of balance between aphid and parasite was clearly recognized by Webster and Phillips. They also realized that temperature changed this balance.

Headlee (1914) attacked the problem again with apparatus suitable for temperature control. He showed that the life cycle of the green bug, *Toxoptera graminum* Rond., was progressively shortened as temperature rose from 50°F (10°C) to 80°F (26.67°C), then lengthened somewhat from 80°F (26.67°C) to 90°F (32.22°C). The parasite of the green bug behaved in a similar manner except that at low temperatures development of the parasite was slowed down more than that of the host. The maximum reproductive power of the host was at 80°F (26.67°C).

Shelford (1926) again studied the balance of Toxoptera and its chief parasite by using Headlee's (1914) data for rate of development and Glenn's (1909) for the threshhold of development. Shelford's method was quite similar to that of Sanderson (1910) and Krogh (1914), but he used a theoretical alpha in place of developmental zero and summed up developmental units in place of temperature. A brief explanation is necessary in order to understand Shelford's curves.

If the time required for an insect to complete a stage of growth, for example the pupal, is plotted against constant temperatures at which the growth takes place, the resultant curve is a rectangular hyperbola. The reciprocal of such a curve will be a straight line. At extreme temperatures, both high and low, there are deviations from the hyperbola and also from the straight line reciprocal. Prochnow (1907, 1908) pointed out that the reciprocal curve was sigmoid rather than linear. What appears to be a straight line is in reality but a segment of a more complicated type of curve that approaches a straight line at medial temperatures. Later workers, among them Friend (1927), Janisch (1925, 1927, 1930) in a series of papers, and Cook (1927) have observed that the reciprocal of the temperature-developmental curve is not linear.

However, the straight line reciprocal is still a valuable method in temperature work. Shelford (1926) compared the development of *Toxoptera graminum* Rond. and its parasite *Lysiphlebus tritici* Ashmead by comparing the number of developmental units required. He states, (p. 285) "The developmental unit is the difference in amount of development taking place in one hour at a given medial temperature, and at another medial temperature one degree higher as shown by the time to complete the stage. Medial temperature is that range of temperature within which the increase in development is directly proportional to the rise in temperature."

Medial temperatures may also be defined as those temperatures at which the reciprocal of the developmental curve is a straight line. Shelford then calculated from Headlee's (1914) data that the developmental total of the host was 4,320 units and of the parasite 5,280. From data of Glenn (1909) the threshold of development for the host is 1°C and the parasite 2.2°C. At 20°C (68°F) a parasite would develop in 264 hours; a host, in 240 hours. During the time of development of the parasite, the host would produce 26.4 individuals. The parasite once emerged could infest 22 aphids (Hunter 1909) in two days. But in the meantime the host would have produced more offspring, making a total of 31.2 or 9 individuals ahead of the parasite. Theoretically it is impossible for the parasite to overtake the host provided neither had unfavorable factors to overcome. If this relation were stated in terms of Chapman's biotic potential, one would say that the biotic potential of the host was greater than that of the parasite.

Ruzička (1925) recorded an interesting case of host-parasite balance and its relation to weather in the nun moth, *Porthetria monacha*, Linn. and its parasites. If the spring temperature is high and humidity is low, the host is favored. If the spring temperature is low and especially if the humidity is high the parasites, both Tachinidae and fungi, increase at the expense of the host. Further discussion of Ruzička's work will be given in the section on the differential effect of humidity on host and parasite.

According to the researches of Bremer (1928) and Blunk, Bremer and Kaufmann (1928), environmental factors, especially temperature, affect the host-parasite balance of the beet-fly, *Pegomyia hyoscini* Panz., and its parasites, *Opius filvicollis* Thoms. and *Phygadenon pegomyiae* Habern. Cool weather retards the parasite more than it does the host. Thus during a cool summer the beet-fly gains rapidly on its parasites. In warm weather the parasites gain rapidly on the host. *Pegomyia hyoscini* has three generations a year. It appears to be cold-hardy both to the intensity and the quantity factors of low temperature.

Barnes (1930) found that the parasites of the gall midge, *Rhadophaga heterobia* H. Lw., were less cold-hardy than the midge itself. The percentage of survival of both *Dasyneura alopecuri* Reuter and *Dasyneura pyri* Bouché, after exposure to low temperature, was less than their host. This conclusion was based largely on material collected in the field.

4. *Differential Action of Humidity on Host and Parasite*

Various insect diseases, notably the polyhedral disease of the nun moth, *Porthetria monacha*, Linn. are favored by high relative humidity. Ruzička (1924) (1925) found that there was a certain periodicity in outbreaks of this moth. This periodicity was associated with cyclic weather changes. The weather cycles did not operate as strongly on the nun moth itself as on its diseases and parasites. Low relative humidity favored the moth; high relative humidity its biological controls. The polyhedral disease was the most important of the diseases; *Parasetigena segregata*, a tachinid, the most important of the parasites.

Hefley (1928) showed that as the relative humidity increased from 0% to 100%, the viability of the hawk moth pupa, *Protoparce quinquemaculatus* Haw., decreased from 100% to 42.44%. The percentage of survival was only 44.44 at 73.4% relative humidity. The viability of the parasite, *Winthemia quadripustulata* Fab., increased steadily from 33.4% at 7.1% relative humidity, to 100% at 73.4% relative humidity. At humidities greater than 73.4% emergence of the parasite was practically 100%. In other words a low relative humidity was favorable to the host and a high one to the parasite. Relative humidity was controlled by means of the salt solution method of Headlee (1914).

Thus there is, with a number of insects, a certain balance between host and parasite. This balance or equilibrium may be considered as kinetic rather than static. Temperature or other environmental factors may shift the equilibrium now in favor of the host, now in favor of the parasite. In fact, in many cases weather favorable to insect outbreaks may be merely weather unfavorable to parasites. In addition to the physical factors of the environment which affect host-parasite balance are certain biotic factors. Among them is one which apparently has been overlooked; namely, competition between parasites.

5. *Host-Parasite Balance as Affected by Competition between Parasites*

If more than one species of parasite attacks a host, do the damages of the parasites produce a cumulative effect or does the competition between parasites give the host a chance to escape unharmed? Important as is this problem, both in theory and practice, there is little data upon it. Often more than one parasite has been introduced to control a noxious insect. Usually a second parasite has been added before the host-parasite balance of the first parasite and its hosts was fully determined. Pemberton and Willard (1918) found that the total parasitism of the fruit-fly, *Ceratitis capitata* Wied., decreased when *Diachasma tryoni* Cam., a less efficient parasite, was added to the very efficient parasite *Opius humilis* Silvestri. Larvae of *Diachasma tryoni* Cam. killed the larvae of *Opius humilis* with their sharp mandibles. There was also a difference in winter survival of the two parasites. *Opius humilis* survived the winter better than *Diachasma tryoni.*

B. Literature on the Insects Used in This Study

1. *The Original Habitat of Ephestia kühniella Zeller*

One of the most effective means of evaluating the results from an experimental study of an insect is to compare the results obtained with those under field conditions, especially in the natural habitat. Unfortunately, the original home of neither *Ephestia kühniella* Zeller nor *Microbracon hebetor* Say has been definitely ascertained.

Ephestia kühniella Zeller was found in Halle, Germany, in 1877 by Kuhn and described two years later by Zeller, as from the United States. In 1881 Snellen recorded the pest from Holland. In the plate which accompanies the article, both a pink and a yellowish type of larva were figured. This is of interest since the writer has found that the pink larvae are not as good food for the *Microbracon hebetor* as are the yellowish larvae. In 1884 Preudhomme de Borre found *Ephestia kühniella* in Belgium, where it was doing damage in a vermicelli factory. By 1886 it was reported in England. Klein (1887) observed that an ichneumonid parasite controlled the moth. Eleanor Ormerod

(1889) believed that this insect had been introduced into England from the Mediterranean ports, where it was a serious menace. Chittenden (1909) considered that the Mediterranean flour moth was causing more complaints than all the rest of the mill pests put together. Both C. V. Riley and Chittenden thought that the pest might have originated in Australia.

In 1893 Danysz made a careful study of the history and distribution of *Ephestia kühniella,* and came to the conclusion that it was not introduced into Europe from America. Danysz thought that the pest had been known as early as 1840 in Paris and had probably been in Constantinople in destructive numbers as early as 1872. Thus, he concluded that Ephestia had first become distributed widely and then secondarily become a pest. Fölsch (1926) agrees that Danysz (1893) was right in this conclusion. Fölsch believed that *Ephestia kühniella* was originally a feeder on honey and an inhabitant of wild bees' nests. Zacher (1922) thought that the pest originated in Central America or Mexico. Lebedev (1929) thought that the moth was indigenous to Mesopotamia or Southeastern Armenia before it became widely distributed.

Thus, the original home of the Mediterranean flour moth has been variously placed in North America, Central America, Europe, Asia, and Australia. To the author's knowledge no one has attempted to place it in Africa. One means of discovering the native home of *Ephestia kühniella* would be to find where it is in equilibrium with its parasites and is controlled by them.

2. *Parasites of Ephestia kühniella*

The parasites of the Mediterranean flour moth include several species of Hymenoptera, a mite, several bacteria, and a few Protozoa. Most of the parasites are not specific for *Ephestia kühniella,* but attack other hosts. A simple list of the parasites will suggest something of the complexity of the balance of *Ephestia kühniella* under conditions where it is freely preyed upon by its enemy organisms.

Parasites are listed in approximate order of their discovery:

Hymenoptera—*Chremylus rubignosus* Hal.
Microbracon hebetor Say.
Mesostenus gracilis Cresson.
Omorga (Campoplex) frumentaria Rond.
Apanteles nephopterieus Packard.
Nemeritis canescens Gravenhorst.
Trichogramma evanescens Westwood.
Trichogramma minutum Riley.
Angita incipiens Walley.?

Acarina —*Pediculoides ventricosus* Newport.

Bacteria —*Bacillus thuringensis* Berliner.
Micrococcus ephestiae Mattes.
Bacillus agilis Mattes.

Protozoa —Unnamed sporozoan reported by White (1927).
Thelohania ephestiae Mattes.

Almost as soon as *Ephestia kühniella* was considered as a destructive insect, parasites were reared from it. In 1887 Klein reported a small ichneumonid parasitic on Ephestia larvae. Bridgeman (1889) did not see the parasites of Klein, but reared *Chremylus rubignosus* Hal. from Ephestia larvae.

In 1884 Kirby found that in some cases a little parasite which he called *Bracon brevicornis* Wesmael was able to control Ephestia. He reared the parasite from *Ephestia elutella*. This *Bracon brevicornis* was the parasite known today as *Microbracon hebetor* Say. Johnson (1895) mentions three parasites of *Ephestia kühniella*, among them *Microbracon hebetor*.

In addition to *M. hebetor*, Johnson mentioned *Omorga (Campoplex) frumentaria* Rond. and *Mesostenus gracilis* Cresson. About the same time Baker (1895) reared *Apanteles ephestiae* from Mediterranean flour moths that had been feeding on honey combs. Muesebeck (1926) considered that the *Apanteles ephestiae* of Baker was the same species as *Microgaster nephotericis* of Packard, which parasite had been reared from *Vitula edmansii* fed on honey combs. If Muesebeck's synonomy be correct, then *Apanteles (Microgaster) nephotericus* Packard is not a specific parasite for *Ephestia kühniella*.

Johnson (1895) reported *Omorga frumentaria* Rond. attacking *Ephestia kühniella*. Kornauth (1917) again reared this species from Ephestia and suggested that the parasite might be a valuable aid in controlling the pest. He called the parasite *Campoplex frumentarius* Rond. Back (1920) considered Omorga (Campoplex) Rond. a valuable secondary or supplemental control for *Ephestia kühniella*, but believed it was not as effective as fumigation or heat treatment.

Another parasite of the Mediterranean flour moth which has attracted considerable attention of late years is *Nemeritis canescens* Gravenhorst. According to Smits van Burgst (1921), the parasite is not as widely distributed as the host. Among other workers Krüger (1920) in Germany and Candura (1928) in Italy have described the life history of *N. canescens* and evaluated its importance as a control measure against *Ephestia kühniella*. The parasite is handicapped by a higher temperature threshold of development than the host. The parasite is unable to feed on young host larvae. Voinovskaya-Kriger (1927) found that *N. canescens* could have as many as 15 generations a year at temperatures of 20-25°C. Daviault (1930) found that the reproductive power of the host for a single generation was 2.5 to 3 times that of the parasite, but that the parasite had two generations to the host's one. Diamond (1929) thought that in a Canadian environment *Nemeritis canescens* would be a valuable supplemental control measure.

Stored products insects of various species, among them *Ephestia kühniella* have frequently been used to rear the egg parasites, *Trichogramma evanescens* Westwood and *T. minutum* Riley. Such work has been done largely with a view to rearing large numbers of parasites for release on the codling moth, *Carpocapsa pomonella* Linn. Undoubtedly there are data which would show the efficiency of the egg parasites against Ephestia, were they to be considered from the Ephestia viewpoint. Schulze (1926) reported that the fecundity of parasites reared on Ephestia was less than when reared on Carpocapsa. Evans (1930) reported *Ephestia kühniella* as furnishing good nourishment for both species of Trichogramma.

A parasite which may be more or less incidental is the mite, *Pediculoides ventricosus* Newport, observed by Herfs (1926). Since this mite occurs on *Isosoma grande* Riley and *Sitotroga cerealella* Ol., it is not surprising that it has been found on *Ephestia kühniella.*

Walley (1929) described *Angitia incipiens* as a new species. This hymenopteron may be a parasite of *Ephestia kühniella* since that moth was being reared in the next room during his observations.

The effect of the bacterial and protozoan diseases on *Ephestia kühniella* is very marked. Whoever undertakes to rear *E. kühniella* in large numbers must sterilize the food or isolate a few larvae in each rearing jar, and take other sanitary precautions if he wishes to keep his culture going. In other words Ephestia is controlled by disease under certain conditions. Berliner (1915) described *Bacillus thuringensis* from *E. kühniella* and reported on its pathogenicity. White (1927) found that in certain cases *B. thuringensis* infection resulted in 100% mortality among flour moth larvae. White also reported a sporozoan which attacked the fat body. Externally this disease made itself known by black spots on the larvae. Whiting had found the protozoan disease in cultures which he was raising for genetic experiments and had sent material to White for diagnosis. In 1927, Mattes described two species of bacteria, *Bacillus agilis* and *Micrococcus ephestiae,* from the Mediterranean flour moth. *B. agilis* was not strongly pathogenic except under certain conditions. It was most frequently associated with *B. thuringensis. Micrococcus ephestiae* Mattes was definitely pathogenic. The microsporidian, *Thelohania ephestiae,* was a disease producer. Mattes described the parasite in 1927. In 1928 he made an extensive research on the morphology, life history, and pathogenicity of the species. *Thelohania ephestiae* may be either severely or only mildly pathogenic, depending on the number of organisms in the host. *T. ephestiae* occurs in the fat body chiefly. Small foci of the disease are also found in the heart and in the intestinal musculature. The means of transmission does not seem to be *per os.* It may be carried by the sting of parasitic wasps. Mattes (1928) also reported the presence of polyhedral disease in *Ephestia kühniella.*

The complete picture of the host-parasite balance of *Ephestia kühniella* with all its insect parasites and diseases is indeed complicated by the non-specificity of many of the parasites. However, the problem investigated here will not be the complete host-parasite balance of all the parasites, but rather the simple one of a single parasite and the Ephestia host.

3. *Evaluation of Microbracon hebetor Say as a Control for Ephestia kühniella Zeller*

Buchwald and Berliner (1910) considered that *Microbracon hebetor* Say was a valuable aid in control of the Mediterranean flour moth. Back (1920) mentions two parasites of the Mediterranean flour moth which are *Microbracon hebetor* Say and *Omorga frumentaria* Rond. He thinks that these parasites are only accessory controls and that heat treatment or fumigation must be relied upon for effective control.

Hase (1922, 1925) made an attempt to evaluate the work of *Microbracon hebetor* in controlling the Mediterranean flour moth. He let loose 100 *M. hebetor* and 500 *Ephestia kühniella* in a space of 9,264 cubic meters which had a surface of 387,348 square meters. He recovered 129 host larvae and of these 70 were parasitized. Later he conducted similar experiments in which he found a parasitism of 50% to 60%. From the concentration of *Microbracon hebetor* necessary to parasitize 50% of the flour moth caterpillars, he calculated that 20,000 female *M. hebetor* per 1,000 cubic meters would be needed for complete control. Hase did not reckon on any cumulative effects of several generations of parasites, but only on one. However, Muir (1913) has shown that a parasite may be overtaking the host when the host is apparently gaining.

The early work of Hase on *Microbracon hebetor* was published under the name of *Habrobracon brevicornis*. In order to follow the literature on this species better, a brief review of the synonomy is given.

4. *Synonymy of Microbracon hebetor Say*

As a result of taxonomic study there is a long list of synonyms including some synonomy that has been introduced because of the confusion between the two species *Microbracon (Habrobracon) brevicornis* Wesmael and *Microbracon (Bracon) hebetor* Say.

The chief references to the literature on the synonomy of *Microbracon hebetor* Say follow:

Bracon hebetor Say. Bost. Jour. Nat. Hist. Vol. 1: 1836. P. 252.

Bracon dorsator Say. Bost. Jour. Nat. Hist. Vol. 1: 1836. P. 253.

Bracon brevicornis Kirby. Trans. Ent. Soc. London. 1884. P. xxxi. Marshall. Trans. Ent. Soc. London. 1885. P. 24. Pl. 1. Fig. 1a and 1b.

Bracon juglandis Ashmead. Proc. U. S. Nat. Mus. Vol. 11, 1889 (1888). P. 621.

Habrobracon hebetor Johnson. Ent. News. Vol. 6. 1895. P. 324.

Bracon (Habrobracon) honestor Riley and Howard. Insect Life. Vol. 7. 1895. P. 428. Misprint for hebetor, corrected in index.

Habrobracon beneficientor Viereck. Proc. U. S. Nat. Mus. Vol. 40. 1911. P. 182.

Habrobracon brevicornis Cushman. Proc. Ent. Soc. Wash. Vol. 16. 1914. P. 101.

Habrobracon juglandis Cushman. Proc. Ent. Soc. Wash. Vol. 24. 1922. P. 213.

Microbracon hebetor Muesebeck. Proc. U. S. Nat. Mus. Vol. 67. 1925. P. 31.

Although Say's original types of *Bracon hebetor* and *Bracon dorsator* have been lost, Muesebeck (1925) thinks there is little doubt from the description that these species are both identical with the later *juglandis* of Ashmead. The type of *Habrobracon beneficientor* is still preserved in the museum. The material of Viereck appears identical with the *juglandis* of Ashmead.

One of the difficulties involved in the study of *Microbracon hebetor* Say is the extreme variability of the species. Individuals may vary from almost pure yellow to pure black according to the temperature at which they were reared and also their genetic constitution. There is considerable variation in size, those reared at low temperature being larger and heavier as a rule than those reared at high. Variation in the number of segments in an antenna also occurs. Some individuals may have more joints in the left antenna than the right, or vice versa.

5. *Influence of Physical Factors on Microbracon hebetor Say*

Temperature controls the length of development, which may be 7-8 days from egg to adult at 33-36°C or 16-20 days at 18-21°C according to Hase (1922). This worker never observed eggs to hatch at a temperature lower than 16°C. He observed also that the life length of the adult varied with the temperature. Whiting (1921) observed variation in the period of development correlated with temperature. He also found differences in color, which had been observed by Hase and others. Some of these color gradations were due to temperature and some to heredity. Schlottke (1926) made a very careful study of the influence of temperature on size and color of *Microbracon hebetor* Say. He found that at low temperatures the adults were larger than at high temperatures. Light colored or yellow individuals occurred at high temperatures and black individuals at low temperatures. The places where pigmentation was last to disappear were at the muscle attachments. Schlottke also found that as the temperature was lowered, the period required for each stage of the insect to develop, i.e., egg, larva, or pupa, was

lengthened in the same proportion. Hase (1922) observed that in general high temperature did shorten each stage in the same proportion, but that spinning the cocoon was one process that could not be shortened beyond a certain point.

Doten (1911) observed that *Microbracon hebetor* was extremely resistant to low humidity and starvation. The females could live for over four months at zero centigrade without food. With food they could live even longer. Whiting (1921) used this cold-hardiness to the quantity factor of low temperature in his work. When caterpillars were not available as food, he stored the wasps in ice boxes.

6. *Effect of Physical Factors on Ephestia kühniella Zeller*

Almost as soon as *Ephestia kühniella* was known as a noxious insect, observers noticed that it developed faster at high temperatures than at low. The literature on this pest is enormous. It attracted the attention of both entomologists and millers upon its arrival in a given locality. In addition the Mediterranean flour moth has been used in certain genetic studies.

Johnson (1894) found that in summer the egg stage was 7 to 9.5 days long, the larva 35-49.5, and that of the pupa was from 10-15 days. The period from the time the egg is laid to the emergence of the moth is 59.5 to 68.5 days. Washburn (1904) found that exposure to 3-5°F for 3-6 days killed eggs and larvae. Much of Washburn's extensive paper was copied from the work of Johnson (1894) and that of C. V. Riley (1889). Dean (1913) observed that 116°F (46.66°C) was fatal to adults, 118°F (47.77°C) fatal to larvae and 120°F (49.44°C) to eggs. He also found that at 80-90°F (26.67-32.22°C), the life cycle from egg to adult was 9 weeks. Goodwin (1914) reported that larvae were killed at 43 to 46°C, and the eggs at 47.5 to 48°C, or about the same temperature. Both of these workers also observed that sudden changes in temperature were especially dangerous to the Mediterranean flour moth.

Janisch (1925) used eggs of the Mediterranean flour moth as material to illustrate the so called "exponential law" of development. Hase (1927) used over 300,000 eggs in his experiments. He found that eggs did not hatch above 34.4°C. Incubation time was the same for eggs laid from 1 to 3 days after mating, but eggs laid later took longer to develop. Exposure of eggs to low temperatures seemed to retard development in proportion to the length of exposure. From these results Janisch came to the conclusion that any departure from the optimum, however short, retarded development.

A paper by Dieuzeide (1926) is of special interest because he studied *Ephestia kühniella* from the warm, dry climate of Algeria. He found all stages to be killed by a high temperature of 118-125°F (47.77-51.66°C) or by a low temperature of 20-30°F (-2.66°C to -1.11°C).

Vukasovitch (1929) observed that the egg stage required 4-14 days depending on the temperature. The pupal stage was 12-13 days at 23°C and 7-10 days at 28°C. More eggs were deposited in weak light than in darkness or full light, the numbers being respectively 206, 132, and 198. Vukasovitch found that the moths laid 171 eggs in a damp atmosphere and 225 in a dry one. The sensitivity of moths to low humidity seems hardly in keeping with this last result which may arise from the number of moths being too small to obtain a fair average. It is also possible that the relative humidity could be fairly low, and yet the absolute humidity quite high.

Ephestia prefers damp food to dry. In fact in dry, sound, stored products the larvae have difficulty getting a foothold. However, once flour or meal is infested, the larvae increase its humidity and prepare the way for more infestation. An interesting case of moisture being the determining factor in food choice was observed by Zacher (1928) and Van Emden (1929). Zacher (1928) found the larvae attracted to nut chocolate, but not by sweet or milk chocolate. Van Emden (1929) found that nut chocolate had a higher moisture content than the other varieties and it was this content that attracted the larvae. In all probability the attraction of larvae to food is more complicated than a pure moisture relation, but moisture does play a part.

Doten (1911) found that the optimum for the Mediterranean flour moth was 27-30°C and that the adults were very susceptible to low humidity. Whiting (1921) made the same observation concerning humidity and also found that the optimum temperature was comparatively high.

Wadsworth (1919) found that moths avoided air currents and light when laying their eggs. He also observed that the moth laid most of its eggs toward the end of its life, which lasted some 21 days. Other writers have observed oviposition during the first day after emergence. It is possible that Wadsworth was dealing with a race that differed in this respect from others which have been studied elsewhere. For example Burkhardt (1919) found that at temperatures from 18-22°C the moths copulated in 12-15 hours after emergence. Oviposition lasted from 4 to 7 days, but most frequently 6 days. The number of eggs laid varied from 75 to 229. Adults live 8-13 days. Males never lived longer than 10 days. At 18-22°C, the egg stage lasted 6.5 days to 7 days. Development took place as low as 8°C, but was greatly retarded. At 8-9°C the larval stage required from 122 to 170 days to develop.

Whiting (1921) used *Ephestia kühniella* as material for genetic research and also as food for the parasite, *Microbracon hebetor* Say. He found that it was possible to bring a generation of flour moths through in 5 weeks at 30°C. He observed, as did Doten earlier, that the adults of the Mediterranean flour moth were very susceptible to low humidity.

Bremer (1926) observed that Mediterranean flour moths generally emerged in the afternoon or evening if kept in a lighted room. If pupae

were kept in light by night and in darkness by day, they emerged in the early morning. Moths from pupae kept in constant darkness emerged about like those kept in normal alternation of day and night.

Brindley (1930) found that at 30°C and 70% relative humidity the larval stage required an average of 41 days for completion. The increase in weight of the larva plotted against time forms the usual sigmoid growth curve.

C. Summary of the Literature Discussion

The host-parasite balance is capable of quantitative treatment and, in principle at least, the problem may be stated in terms of mathematics and solved for unknowns if there are sufficient experimental data on host and parasite. The efficiency of a parasite and the possibility of its exterminating the host has been definitely evaluated for the "green bug," *Toxoptera graminum* Rond. and its parasite, *Lysiphlebus tritici* Ashmead. In this case the parasite is unable to control the host.

Environmental factors may accelerate the development of the host more than the parasite or the parasite more than the host. The host and parasite may differ in cold-hardiness to either the intensity or quantity factor of low temperature.

Finally, the life history and ecology of the host and parasite under consideration, namely *Ephestia kühniella* and *Microbracon hebetor,* have been discussed. The threshold of development of *Ephestia kühniella* is about 8°C, the maximum temperature for egg development is about 34.4°C. Larvae are capable of withstanding moderately low temperatures for several months. Eggs seem to be injured by low temperatures, especially when there are sudden changes from a cooler to a warmer temperature. The maximum temperature at which *Ephestia kühniella* will survive differs with different stages. The eggs are the most heat resistant, a temperature of 47-48°C being required to kill them. All stages of the insect are retarded by low temperature and hastened by high temperature. Development from egg to egg may require from 35 days, the shortest period, to somewhat over 250 days. At the same temperatures different observers have found different time requirements for development. When the observations have been made under carefully controlled conditions, these various results may be interpreted as indicating genetic differences in the material used. *Ephestia kühniella* is sensitive to moisture and light.

Microbracon hebetor Say is a parasite of all the common species of Lepidoptera infesting stored products. It is favored by high temperatures and is able to withstand extremes of dryness beyond those which *Ephestia kühniella* is able to bear. Not only length of development, but also color and size of this parasite are dependent upon temperature.

The original home of neither the Mediterranean flour moth nor its parasite is known. Lack of accurate knowledge of the native habitat of *Ephestia kühniella,* to a certain extent makes a check on laboratory experiments difficult. One cannot judge how near he is approaching "natural conditions" if he does not know what they are. However, the special micro-climate of the mill or warehouse can be approximated.

Type of food as a distinct factor has not been discussed since Richardson (1926) has already made an extensive study of food relations.

III. METHODS AND MATERIALS USED IN THESE EXPERIMENTS

Two genetic strains of the Mediterranean flour moth, *Ephestia kühniella* Zeller were used in these experiments. Pure lines of *Microbracon hebetor* Say were not kept, but there was little variation between the reactions of different individuals to temperature. The females were almost pure yellow at 30°C and almost black at 16.5°C. The stock was remarkably free from abnormal individuals. No twisted wings, white eyes, or stumpy antennae were observed. Undersized adults were produced only when too many parasite larvae were placed on one host caterpillar or when the parasites fed on pink Ephestia larvae.

The temperature studies on *Microbracon hebetor* were begun at the Biologische Reichsanstalt in Berlin-Dahlem, continued at Woods Hole, Massachusetts, and at the University of Pennsylvania. The equipment for the control of temperature here described is that at the Anstalt in Dahlem. With the exception of the series incubators, the apparatus at Woods Hole and at the University of Pennsylvania is based on the same principles as that at Dahlem.

Stocks of both *Microbracon hebetor* Say and *Ephestia kühniella* Zell. were also furnished the writer by Dr. P. W. Whiting of the University of Pittsburgh. Both species reacted differently to temperature from those used at Dahlem. Detailed studies have been carried out on *Ephestia kühniella.* The Ephestia from Dr. Whiting develops faster and has a higher optimum than the stock used in Dahlem. In all cases Ephestia larvae were fed on rolled oats, since the experiments in Dahlem were performed on larvae fed on this material.

The temperature control apparatus at Dahlem consists of incubators in series, one at low temperature, and the other at high, and of individual incubators. There was no circulating air current in the incubators. For the insects in question, the lack of air circulation was not a serious fault, but for insects eating green food this factor has been found to be a grave source of error. The low temperature series was kept in a cellar room which was itself at low temperature. The temperatures of the individual compartments in the

low series were 16.5°C, 11.5°C, 9°C, 8°C, 5°C, 3.5°C, 2.2°C, and 0.9°C. The average deviation from these temperatures was ± .5°C and the greatest deviation was ± 1°C. Thermometers were read twice daily. The high temperature series was less constant than the low. In fact there were several compartments in the middle of the series with a temperature that varied as much as 4°C. The source of heat was gas. Temperatures of 38°C, 36.5°C, 32°C, 30°C, 28°C, 27°C, 25.4°C, 23.5°C, and 19°C were maintained. The first three compartments on the high side and the first two on the low side did not vary more than ± 1°C. The others could be used only for rough comparative study to block out limits of duration of different stages. These limits could then be determined more accurately with individual incubators. In one series of experiments, the compartment at 36.5°C fell to 34°C. Since the temperature remained constant at 34°C, advantage was taken of the change to rear two generations of Microbracon and a generation of its host. In all incubators, but in the series apparatus especially, it is important that the thermometer used for reading temperature be at the same level at which the insects are kept. The thermometers at the tops of the series compartments could not be used as fair indicators of the temperatures inside.

Individual incubators kept at 10°C, 16°C, 25°C, 27°C and 30°C, were used. Two rooms, one kept at 18-19°C, and the other at 15°C during the early part of the year, were also available. Low temperature is much more difficult to control than is high temperature. The best results were obtained by placing an incubator in a room colder than the temperature desired. A room at 4°C was used for the 10°C and the 16°C incubators.

Since the maximum and the minimum temperatures at which host and parasite are able to develop is very important, especially if they differ in the two forms, these limits were determined. At temperatures near the developmental threshold, a very small numerical advantage of either host or parasite may result in a striking difference in their numbers in a few generations.

Modifications of the formula used by Chapman (1925) and Thompson (1922) have been used to calculate reproductive potentials and host parasite balance.

IV. RESULTS OBTAINED

A. Rate of Development and Reproductive Potential of Host and Parasite

The reactions of different stocks of Mediterranean flour moths and their parasitic wasps to temperature, showed that the stocks did not behave alike. In the flour moths, there were at least two strains, one of which developed from egg to egg in 35-37 days at 32°C, and the other required 62-65 days. Different stocks of Microbracon also reacted differently to temperature. In the experiments reported here only the healthy Dahlem stock was used. The

samples of the Mediterranean flour moth obtained in Germany all belonged to the slow type. Those from the United States contained both slowly and quickly developing strains. It is not known how life length is inherited in these moths, but since food and temperature were kept constant, differences in period of development were probably genetic. The developmental zero of the slow type is about 8°C; of the quick type, 10°C. It is very difficult to get moths of either type to lay eggs below 15°C. The number of eggs laid per female seems to be about the same in both stocks. Both types had a large percentage of sterility under the conditions in which they were reared. The annual reproductive potential of the two strains differs enormously, largely on account of the number of generations a year. A comparison of the two strains with reference to duration of development and number of generations a year is shown in Table 1.

TABLE 1. Development of *Ephestia kühniella*.

Temperature	Time in days required for fast strain	Time in days required for slow strain	Number of generations per annum of fast strain	Number of generations per annum of slow strain
32° C.	35–37	62–65	10.4–	5.8
30° C.	41–43	66–68	8.9–	5.5
27° C.	43–45	69–72	8.5	5.3
25° C.	50–53	79–81	7.3	4.6–
20° C.	63–65	99–103	5.8	3.7–
15° C.	85–90	135–157	4.3	2.6–
10° C.	140–175	205–243	2.6	1.08
8° C.	cannot develop	250–270	...	1.04

The number of generations a year has been calculated from the minimal number of days required for development at each temperature. At lower temperatures especially, there is considerable variation in the time required from egg to emergence. Since overcrowding also produces a spread, it is believed that increase in variation, with this particular species at least, may be an indication of an unfavorable environment.

The effect of temperature on the development of *Microbracon hebetor* is very marked. The threshold temperature required for development is higher than that of the host. Hase (1922) found that eggs did not hatch below 16°C. The present writer has managed to rear a few eggs at 15°C by keeping them well covered by host larvae and by adding some dry $CaCl_2$ to take up excess moisture. The relative humidity rather than the absolute humidity seems to be the factor involved, since the absolute humidity with saturation at 15°C is less than the absolute humidity at 25°C with a 70% saturation. Females were never observed to lay eggs at 10°C. Black females reared at 16°C could lay eggs at 14.5-15°C but were very sluggish and clumsy in the act of oviposition.

The maximum temperature at which all stages can develop is 36°C. Both

eggs and adults kept at 38°C looked dried. Efforts to rear parasites at 38°C in a saturated atmosphere failed, however. Death at 38°C must therefore have been due to the high external temperature rather than the environmental humidity. However, the insect tissues themselves might have dried out. Investigation is now in progress as to the cause of death from high temperatures.

The rapid succession of generations in *Microbracon hebetor* at high temperatures is very striking. The short life cycle enables this parasite to have as many as 52 generations a year. The time required for development and the possible number of generations a year is shown in Table 2.

TABLE 2. Development and Length of Life of *Microbracon hebetor*.

Temperature	Time in days required for development	Number of generations per annum	Median life length of female in days
36° C	7	52–	3
34° C	8	45.6	6
32° C	9	40.5	9
30° C	10–11	36.5	13
27° C	11–12	33.2	20
25° C	13–14	28.7	23
20° C	19–20	19.2	28
16.5° C	.....	....	55
10° C	.....	....	90

The reproductive potential of an organism depends on the offspring produced each generation, and also upon the number of generations a year. The number of eggs laid per female, the sex ratio of the offspring and the fertility of the eggs laid all influence the reproductive potential. The fertility of imagoes of Ephestia is very low. At temperatures of from 25°C to 32°C, about 70% of the moths prove to be fertile. The percentage of females varies from 52.5 to 57% with a mean of 54%. At 20°C about 65% of the moths are fertile, and at 15°C, the fertility is about 50%. It is difficult for moths to lay eggs at 10°C or 8°C. From 10-12% of the stock that develops at a slow rate will lay eggs at these low temperatures. All stages are able to survive a lower temperature than they require for development.

The reproductive potentials of the Mediterranean flour moth are calculated by multiplying the average number of eggs laid by the decimal representing the sex ratio, and multiplying this product by the percentage of fertile matings at a given temperature. The offspring in a year's time may be represented as follows:

If E = numbers of eggs laid

S = the sex ratio expressed as percentage of females to the total population

F = the percentage of fertile females, i.e., females that lay eggs

G = number of generations per year

Then $(E\ S\ F)^G$ at any temperature is the number of offspring at the end of the year.

The maximum reproductive potential of the Mediterranean flour moth is reached at 27°C. Higher temperatures show a slight falling off in potential. At the lower temperatures the decrease in reproductive potential is not so much due to fewer eggs laid per female as to the small number of females which are capable of laying eggs. The percentage of fertile eggs decreases markedly with fall in temperature also. This loss of fertility is due more to the increase in unfertilized eggs because the males are so sluggish that they fail to mate, rather than to decrease in viability of fertilized eggs. Figure 1 shows the reproductive potential of *Ephestia kühniella* plotted against temperature. Table 3 shows the number of eggs laid, the sex ratio, the fertility

TABLE 3. Reproductive Potential of *Ephestia kühniella*.
Fertility expressed as percentage of females laying eggs

Temperature	Eggs laid per female	Sex ratio	Fertility of fertilized females	Reproductive potential
32° C	181	54	0.70	68.62
30° C	204	54	0.70	77.11
27° C	225	54	0.70	85.05
25° C	200	54	0.70	75.60
20° C	177	54	0.65	62.13
15° C	156	54	0.50	42.12
10° C	67	54	0.10	3.62
8° C	55	54	0.10	2.97

of the female, and the net reproductive potential at the different temperatures studied.

The reproductive potential of the parasite, *Microbracon hebetor* Say, requires a somewhat different formula from that for the host. As a rule, unfertilized females produce males only and fertilized females produce females only. Occasionally there is a biparental male. In calculations involving host-parasite balance, the number of parasites placed on one host must be taken into consideration. If one parasite can kill a host, then any more parasites per host decreases the efficiency of the parasite. It can easily be seen that twenty eggs distributed to kill twenty hosts is more efficient than twenty eggs deposited all on one host. The average number of parasites per host is ten. The efficiency of the parasite for one generation may be considered as its reproductive potential divided by the number of parasites per host. The total annual reproductive potential of the parasite may be expressed by the formula; $(esf)^g$ where

e = number of eggs laid per female

s = sex ratio expressed in the percentage of females to the total population

f = fertility of the females expressed in percentage
q = number of generations *per annum*

In practice the factor f is dropped out in the case of Microbracon because with the stock used there are so few infertile individuals that it was safe to reckon with a fertility of 100%. The maximum reproductive potential of the parasite lies at 27°C. The number of eggs laid per female is less for the parasite than for the host, but the net reproductive potential of the parasite is greater than that of the host at all temperatures above 15°C. The larger reproductvie potential is brought about by a high ratio of females to males and a very high rate of fertility in the females. Under the conditions of the experiment 100% hatches from the eggs of the parasite were the rule and not the exception. Females unable to lay eggs were also very rare. About one female in 3,000 was physiologically sterile. The sex ratio in the tables is 0.75, which figure was obtained by counts of stock populations reared at the various temperatures used. At 15°C the sex ratio changes, largely on account of the sluggishness of the males. Unfertilized females produce males. At low temperatures the male is so inactive that he often fails to mate. When he copulates he does so very clumsily. A similar awkwardness may be observed when the female parasite tries to sting a caterpillar at low temperature. Quite often she fails to hit the larva at all. At temperatures of 25°C or above, the same female is able to paralyze the caterpillar with a quick thrust of the ovipositor. The eggs are not laid in the oviposition puncture, but are deposited under or near the larva. The young larva must crawl to the paralyzed host to obtain its food. If the eggs are laid too far away from the host larva, the young parasite may starve to death before it finds nourishment. At low temperatures the female parasite appears to lay her eggs without much regard to the possibility of the newly hatched larva obtaining food. It is possible that the odor of the host larva is not so apparent at low temperatures as at high. However, the most pronounced effect of low temperature on Microbracon is on the neuro-muscular coördination, including the act of oviposition.

TABLE 4. Reproductive Potential of *Microbracon hebetor*.

Temperature	Eggs laid	Sex ratio	Reproductive Potential	Parasitic efficiency
36° C	90	.75	67.50	6.75
34° C	105	.75	78.75	7.87
32° C	111	.75	83.25	8.32
30° C	114	.75	87.50	8.75
27° C	120	.75	90.	9.00
25° C	113	.75	85.	8.50
20° C	87	.75	65.25	6.52
15° C	56	.50	28.	2.80
10° C	..	...	.....	....

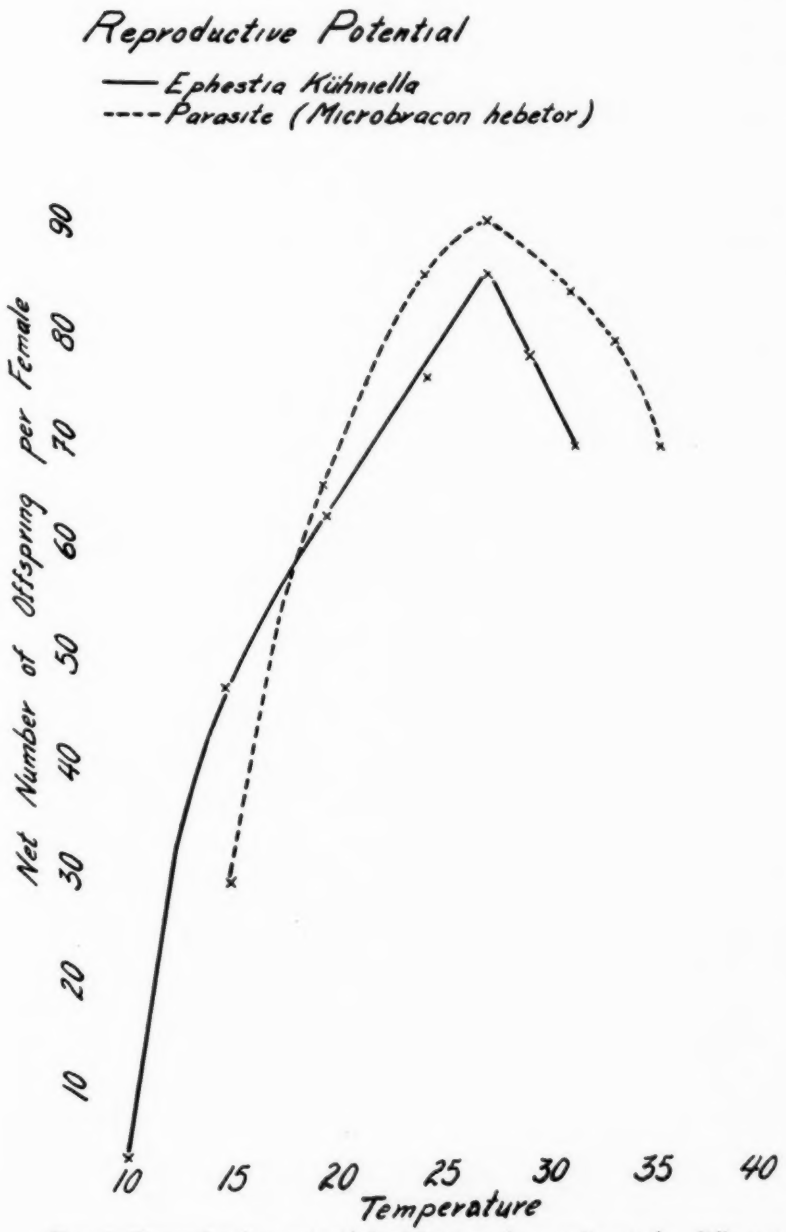

FIG. 1. Reproductive potential of host and parasite at the different temperatures studied.

The effect of temperature upon reproductive potential is shown in Figure 1 and Table 4. In Table 4 the eggs laid, the sex ratio, the reproductive potential, and the parasitic efficiency are shown. The parasitic efficiency is the reproductive potential of the parasite divided by the number of eggs laid per host. The number of parasites per host varies from 1 to 22 with a median value of 10. More than 22 parasites may be started on a host, but some of them will die from undernourishment.

The maximum parasitic efficiency comes at 27°C, at which temperature the host also shows its maximum reproductive potential.

B. Development of the Different Stages of *Ephestia kühniella* as Affected by Temperature

Two strains of *Ephestia kühniella* were used in this study. The fast strain developed from the egg in 35-37 days and the slow one in 62-65 days. Eggs of both strains can hatch at 34°C, but the rate of larval mortality is so high that it is difficult to get a stock culture of sufficient numbers to give significant results.

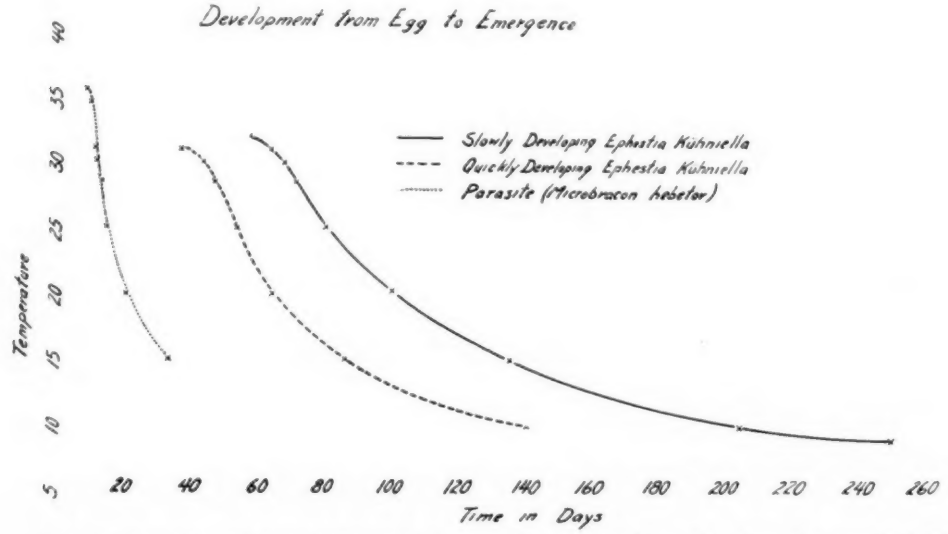

FIG. 2. Duration of development of host and parasite from the time the egg is laid until the emergence of the adult, plotted against temperature.

Since the parasite feeds on the larva only, the duration of this stage is of special importance in determining a host-parasite balance. In the slow strain the larvae live from somewhat over 140 days at 8°C to 53-55 days at 32°C. In the strain which develops quickly, the larval stage requires 53-62 days at 10°C, and 28-29 days at 32°C. The egg stage in the slow strain requires 4 days to develop at temperatures of 27-32°C inclusive. The egg stage in the fast strain requires from 3-27 days, depending on the temperature. The greatest variation with temperature comes in the larval stage. The length of each stage for both strains at temperatures from 8°C to 32°C is shown in Table 5. Figure 2 shows the duration of development in both strains from the time the egg is laid to the emergence of the adult. The type of developmental curve for both fast and slow strains is the same, namely, a rectangular hyperbola of the general type $xy = c$, where x is the time in days required for development, and y the respective temperatures. For the quickly developing strain the constant is 1248; for the slowly developing, 1979. Thus,

the equations for development in relation to temperature differ only by a constant. The curves of development are shown in Figure 2.

The difference between the two strains can best be realized by comparison of their annual reproductive potentials at a given temperature. For the slow strain the annual reproductive potential $(ESF)^G$ at 27°C equals (204) (0.54) $(0.70)^{8.9}$, or 6.24×10^{15}; the fast strain, (204) (0.54) $(70)^{5.5}$, or 2.39×10^{19}. In other words the annual reproductive potential of the fast strain is more than a million times that of the slow strain.

C. Development of the Different Stages of *Microbracon hebetor* Say as Affected by Temperature

The type of temperature developmental curve for *Microbracon hebetor* is quite different from that of either strain of the host. The period of development from egg to emergence plotted against temperature will not give a rectangular hyperbola. The type of curve is probably an exponential curve, the equation of which will be derived in another paper.

The total life cycle of Microbracon is very short. It varies from 7-30 days at temperatures ranging from 36°C to 15°C. The life cycle is characterized by a comparatively short feeding stage, the larval; and by long resting stages, the egg and pupa. The minimum time thus far observed for the egg stage was 12 hours. In the strain used there was slightly more variation in the pupal stage upon change in temperature than in the egg stage. The length of each immature stage of the parasite is shown in Table 5. Figures 3 and 4 also show the effect of temperature on the duration of each stage of the parasite.

The effect of temperature on the life length of the adult is shown in Table 7. If the frequency curve of distribution for the different life lengths were plotted, the resultant curve would be skewed. Therefore the median rather than the mean figures are used in calculations. In almost every group of females, even from a pure line, there are some which die at the end of a day and others which live to extreme old age. At each temperature studied the duration of life of the adult males was less than that of the female.

D. Host-Parasite Balance If the Parasite Is Added to a Culture of the Host Larvae

An inspection of Figures 2, 3 or 4, or Table 5 will suggest immediately the possibility that *Microbracon hebetor* could exterminate its host at high temperatures. At 36°C the host can no longer complete its life cycle but the Ephestia larva can live and even pupate. The eggs of the host are unable to hatch above 34.4°C. At 34°C (not shown in either tables or figures) a few of the hosts are able to emerge, but of the females emerging only 13% are fertile, of their eggs only 69% develop, and of the larvae which hatch only

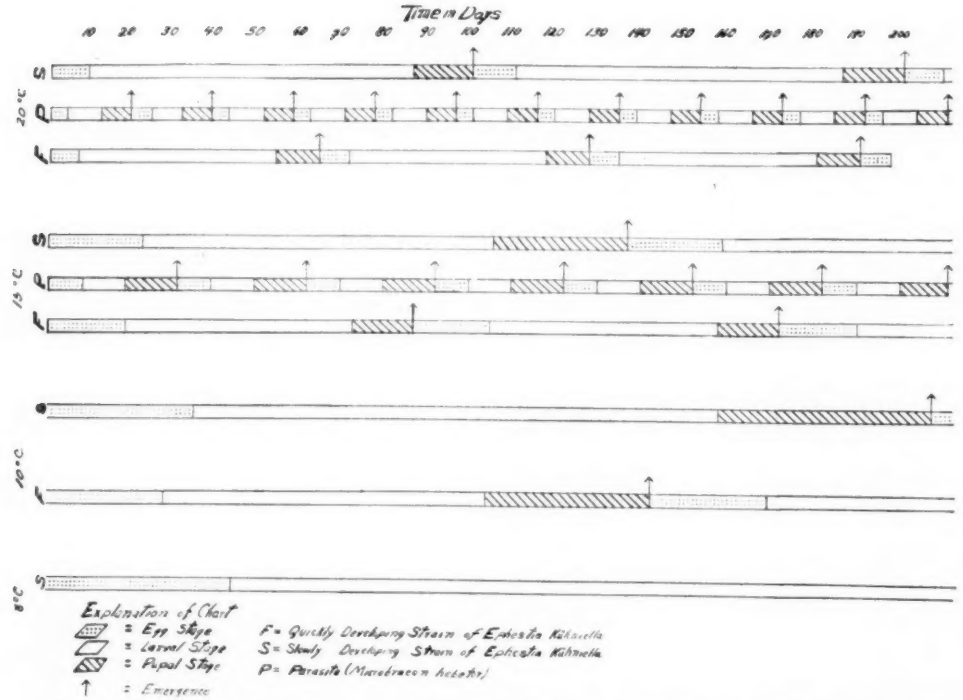

FIG. 3. Length of egg, larval, and pupal stages, and the time of emergence of two strains of host *(Ephestia kühniella)* and one of parasite *(Microbracon hebetor)*.

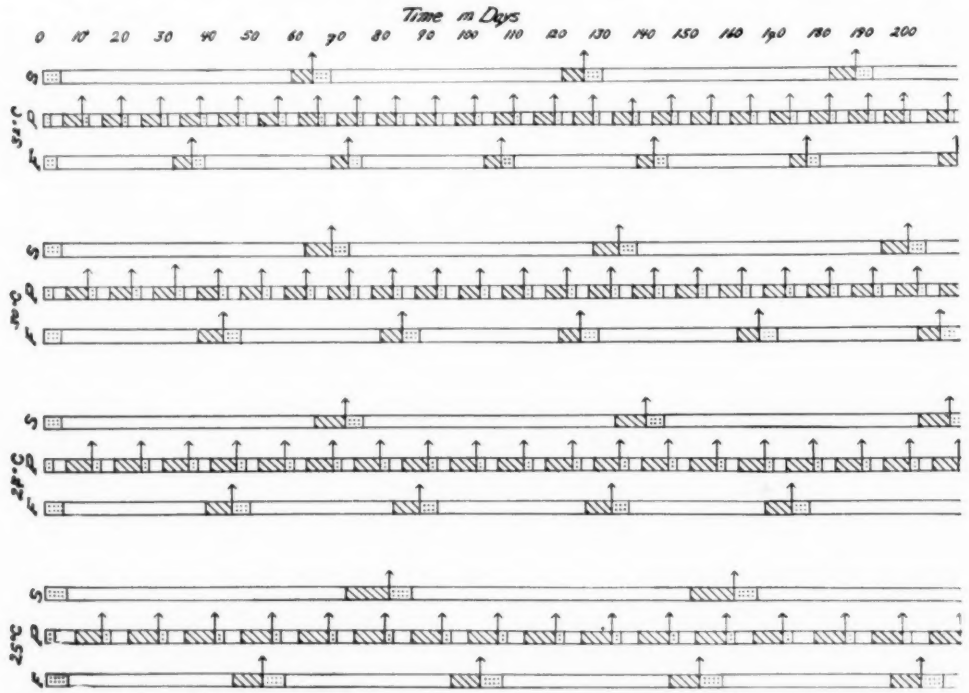

FIG. 4. Length of egg, larval, and pupal stages and time of emergence of two strains of host *(Ephestia kühniella)* and one of parasite *(Microbracon hebetor)*. Symbols used are the same as in figure 3.

40% are able to pupate. The net reproductive potential of Ephestia at 34°C is about 1.5 for the fast strain, and somewhat less for the slow strain, namely 1.2. At 34°C the upper developmental limit of the parasite is 2°C higher than that of the host. Thus, at the extreme high end of the temperature scale the parasite has the advantage.

TABLE 5. Time required for development of host and parasite expressed in days.

Temperature	Development of *Ephestia kuhniella*								Development of *Microbracon hebetor*			
	Slow strain				Fast strain							
	Egg	Larva	Pupa	Total	Egg	Larva	Pupa	Total	Egg	Larva	Pupa	Total
36° C	..	..	..	..	..	..	..	..	1	3	3	7
34° C	..	..	..	..	..	..	..	..	1	3	4	8
32° C	4	53	5	62	3	28	4	35	1.5	3	4.5	9
30° C	4	57	5	66	4	32	5	41	2	3	5	10
27° C	4	58	7	69	4	33	6	43	2	3	6	11
25° C	5	64	10	79	5	38	7	50	2	5	6	13
20° C	9	76	14	99	7	46	10	63	4	8	7	19
15° C	22	82	31	135	18	53	14	85	8	10	12	30
10° C	34	122	49	205	27	75	38	140	..	..	..	..
8° C	43	143	64	250	..	..	..	..	..	..	..	..

At the low end of the temperature scale, however, the host has the advantage since it has a lower threshold of development than the parasite. The fast strain of host is able to complete its development at 10°C; the slow strain, at 8°C. The parasite eggs cannot hatch much below 15°C. In fact the minimal developmental temperature is 14.4°C. The reproductive potential of the parasite at 15°C is very low. When the parasite and the fast strain of host both require the minimum number of days to develop, the host has a larval period of 53 days and the parasite a life cycle of 30 days. The reproductive potential of the parasite is 28. This low reproductive potential is a result not only of fewer eggs laid, but also of a larger proportion of males to females than at higher temperatures, for at 15°C many of the males are too sluggish to copulate. Since the fertilized eggs produce females and the unfertilized males, any factor which decreases the number of matings will affect the sex ratio. The parasite lays on an average 10 eggs per host. Therefore the hosts killed by the first generation of parasites is equal to one tenth of the reproductive potential of the parasite. If 10 female Microbracon are set on 100 Ephestia larvae the number of hosts killed by their offspring at 15°C will be 28; the Ephestia remaining 72. If the larval period of the fast strain of host requires 60 days to develop, the parasite can exterminate the host by the second generation. At the end of the second generation the number of parasites will be 784. These will be able to kill 78.4 hosts or slightly more than the number remaining. If the parasite requires even one more day for development than its minimum, the parasite will not be able to reproduce twice

during the larval period of the host and so will be unable to destroy the host. When host and parasite are as closely balanced as they are at 15°C even small variations become important. Thus, retardation of one day in the parasite development may mean that the host is able to survive parasite attacks. The death of as few as 3 parasite larvae in the first generation will permit the host to survive. If the fast strain of host requires a minimum time to develop and the parasite the minimum also, then the parasite is able to exterminate the host in 2 generations, if there has been no pre-imaginal mortality in the parasite. If more than three larvae of the first generation of parasites die, then the host is able to survive. If the host requires the maximum time for development, and the larva the minimum, then the parasite is able to destroy the host. If the parasite requires the maximum number of days to develop and the host the minimum, the parasite is able to catch up with the host if there is no larval mortality; but not if the mortality among the first generation larvae is greater than three. If both host and parasite require maximum period for development the parasite may exterminate the host.

TABLE 6. Host-parasite balance at the different temperatures used.

Temperature	Larval period of Host in Days		Length of Parasite Life Cycle in Days	Initial number of parasites	Initial number of hosts	Hosts killed by Parasite. 1st generation	Hosts remaining	Parasite potential by 2nd generation
	Slow strain	Fast strain						
32° C...	53	28	9	10	100	83.20	17.80	6952
30° C...	57	32	10	10	100	87.50	12.50	76542.5
27° C...	58	33	11	10	100	90.	10.	81000
25° C...	64	38	13	10	100	85.	15.	72250
20° C...	76	46	19	10	100	76.	24.	57760
15° C...	82-90	53-62	30-40	10	100	28.	72.	*784.00

*If host larvae in fast strain requires only minimum time the parasite can never catch up with it.

The problem to be investigated was not only to determine at what temperatures the parasite could control the host, but how long the parasite would require to gain control. The number of days required for a parasite to exterminate the host is the number of parasite generations required to produce as many parasites as there are hosts, times the length of one generation in days. In these calculations the initial number of host larvae is 100; the initial number of parasites, 10. The parasitic efficiency of Microbracon is represented by the product of the number of eggs laid, the sex ratio and the fertility of the female, divided by the number of eggs laid per host; since less than one female parasite in 3,000 proved to be sterile, the fertility of the females is reckoned as 100%. Also since there was as a rule, no pre-imaginal mortality except at extreme temperatures, no factor is used for death rate of immature stages. If the parasitic efficiency is one tenth the reproductive

potential then the parasite will be able to control the host in 18 days at 32°C, 22 days at 27°C, 26 days at 25°C and 38 days at 20°C. These results are based on figures for minimum duration of development of both host and parasite.

E. HOST-PARASITE BALANCE IF THE PARASITE IS ADDED TO A CULTURE OF EGGS OR PUPAE OF THE HOST

Since the life cycles of host and parasite are not synchronous, the adult parasite may emerge at a time when the host is in either the egg or pupal stage and can furnish no nourishment either for the adult parasite or its offspring. When the parasite emerges at such a time the possibility of its surviving or of exterminating the host depends chiefly on its ability to live over the period of egg or pupa of the host. In other words the parasite must be able to starve during the egg or pupal period of the host and still retain capacity to reproduce itself.

TABLE 7. Comparative life length of adult parasite and stages of host upon which it cannot feed.

Temperature	Life Length of adult parasite	Duration of Egg Stage of Host		Duration of 1st Larval Stadium		Duration of Egg + Pupa of Host		Duration of Pupa + Egg + 1st Instar Larva of Host		Duration of Egg + 1st Instar	
		Slow strain	Fast strain	Slow strain	Fast strain	Slow strain	Fast strain	Slow strain	Fast strain	Slow strain	Fast strain
32° C.....	9	4	3	8	4	9	7	17	11	12	7
30° C.....	13	4	4	8	5	9	9	17	14	12	9
27° C.....	20	4	4	9	6	11	10	20	16	13	10
25° C.....	23	5	5	11	7.5	15	12	26	19.5	16	12.5
20° C.....	28	9	7	12	9	23	17	35	26	21	16
15° C.....	53	22	18	18	12	53	32	71	44	40	36
10° C.....	90	34	27	..	..	..	..	..	..	..	..

If the adult parasite emerges during the egg stage of the host it must not only be able to live through the host egg stage without feeding, but also the first stadium of the host larva, since the first stadium cannot furnish support for the parasite. The parasite does not attack first instar larvae. The period that the female parasite must live without food is shown in Table 7 for each of the temperatures studied.

At 32°C the female parasite has a median life length of 9 days (Table 2 or 7). The duration of egg stage and first stadium larva of the host is 12 days for the slow strain (Table 7) and 7 days for the fast strain. At 32°C

it will be seen that the parasite cannot survive if the adult emerges at the beginning of the egg stage of the slow strain of the host. The parasite can survive the egg stage and the first larval stadium of the quickly developing strain of the host. At 30°C the adult parasite can survive the egg stage and the first instar larva of both the slow and the fast strain of the host. At temperatures below 30°C the adult parasite can outlive both the egg stage and the first instar larva of either strain of host.

However, not only the question of survival, but also the reproductive capacity left after a period of starvation determines whether or not the parasite is able to destroy the host. In the strain of Microbracon used here, the number of eggs laid each day tended to be constant during the life of the female. Thus if the female had a total reproductive potential of 83.25 during its entire adult life, the reproductive potential for one day (if it lived 9 days) would be approximately one-ninth of the total. At 32°C the female Microbracon has an adult life length of 9 days. Since the quickly developing strain of *Ephestia kühniella* will supply no food for 7 days and the adult does not lay eggs on the first stadium larvae, the effective reproductive capacity of the female will be two-ninths of the total for the temperature in question, or approximately 18.5 for one parasite. The average parasitic efficiency for each parasite will be 1.85. If at the beginning of the experiment, there are 100 hosts and 10 parasites, the number of hosts remaining at the end of the first generation of parasites will be 81.50; by the end of the second generation the host will be exterminated. At 30°C the parasite has but one day in which it can lay eggs on suitable host larvae of the slowly developing strain. The duration of the egg stage plus the first stadium larva of the host is 12 days; the life length of the parasite is also 12 days. If the adult parasite is able to lay eggs the last day of its life, the race of parasites can survive. Since the parasite tends to lay the same number of eggs each day during the oviposition period its reproductive power on the twelfth day of its life may be considered as one-twelfth of the total reproductive potential or one-twelfth of 87.50, or 7.29. If there are 100 hosts and 10 parasites at the beginning of the experiment, there will be 92.71 at the end of the first parasite generation; there will be 87.29 hosts left. By the end of the second parasite generation there will be 6378.75 parasites capable of killing off 637.8 hosts, or several times the number of hosts present. If the average effective reproductive potential be only 1.4 the parasite will be able to destroy the host. Practically, if some of the adult parasites leave only one offspring and others two, but the average is 1.4, the host can be destroyed. At temperatures other than those for which the host-parasite balance is here calculated, the parasite can control the host except at 15°C. At this temperature, even under the most favorable conditions, when a food supply is at hand as the parasite emerges, the host-parasite balance is very delicate. When the

effective reproductive potential is decreased, as it is when the parasite emerges during the egg or pupal stage of the host, the host may survive the parasitic attack.

If the parasite emerges at the beginning of the pupal period of the host, it must wait for food not only during the pupal period itself, but also during the pre-oviposition period, the egg stage, and the first larval stadium of the host. In the strains of Ephestia studied the pre-oviposition period was so short that it could be neglected safely in calculations. The duration of the adult life of the parasite is shorter than the total of the aforementioned periods of the slow strain of host except at 27°C. In other words, at temperatures other than 27°C, a parasite emerging at the beginning of the pupal period of the slow strain of host would die of starvation. At 27°C the adult life length of the parasite is just equal to the total duration of pupa, plus egg, plus first stadium larva of the slow strain of the host. If the adult parasite is able to lay eggs on the last day of her life and those eggs survive, the parasite will be able to destroy the host. If the total reproductive capacity of the parasite is 90 at 27°C, and the effective reproductive capacity on the 20th day of the adult's life is one-twentieth of the total, then the reproductive capacity left after the period of starvation would be 4.5. If the initial ratio of hosts and parasites be 100:10, at the end of the first generation of parasites there would be 95.5 hosts left. By the end of the second generation there will be 4,050 parasites able to kill off 405 hosts. If the effective reproducing power of the female parasite is only 1.1, the parasite will be able to exterminate the host, providing there is no pre-imaginal mortality of the parasite. The life length of the adult parasite at the different temperatures studied is shown in Table 7. A comparison of the life length of the adult parasite, with the duration of the periods of the host on which it cannot feed is also shown in Table 7.

If the parasite emerges at the beginning of the pupal period of the quickly developing strain of host it can both survive and destroy the host at temperatures from 27°C to 20°C inclusive. At 32°C and 30°C the adult parasite will die before the host larva is able to furnish food. At 15°C the parasite can survive, but its diminished reproductive power will be insufficient to control the host.

If the parasite emerges during the larval stage of the host, the fast strain has an advantage over the slow one since the fast strain does not furnish nourishment for as long a period as the slow one, and does not permit of as many generations of parasite developing on it. On the other hand, if the parasite emerges during the egg or pupal stage of the host, the slow strain would have a greater chance of surviving parasite attack, since it could overcome the parasite by starving it out. In Table 7 it will be seen that only at 27°C will the parasite be able to overcome the slow strain of host, but at all

temperatures between 27°C and 20°C inclusive it can annihilate the fast strain. Taking the host life cycle as a whole, however, the fast strain does have the advantage, since the larval stage of the host, during which period this strain is benefitted by its rapid development, is longer than the sum of the other stages.

In addition to temperature and the time at which the parasite emerges there are other factors which play a part in host-parasite balance.

F. Humidity as Affecting Host-Parasite Balance

Doten (1911) and others have observed that the adults of *Microbracon hebetor* are extraordinarily resistant to low humidity. The same holds true for the eggs, pupae, pre-pupae, and spun larvae. The only stage which cannot withstand drying is the active feeding larva. It can be seen from the tables, or better yet from Figures 3 and 4, that the larval stage is less than half the whole life cycle.

The host, *Ephestia kühniella,* is quite different. The adults, especially, are sensitive to low relative humidity. It may be that the large percentage of sterility observed in the adults by Whiting (1921) and others was due in part to the low humidity of the breeding chambers. Even under the most carefully controlled conditions there is a large amount of sterility in the females. Excess dryness in the pupal period generally induces an increased number of crippled and deformed adults even in healthy stocks. Some of the abnormal adults are unable to clear themselves of the abdominal skin of the pupa. Such adults are unable to reproduce. The larva is able to withstand very little drying. It prefers damp food. The egg can probably withstand dessication better than any of the other stages, but even it seems to be affected by unfavorable low humidity. Young Microbracon larvae are especially sensitive to high relative humidity. Saturation at 15°C will kill them, but will not kill freshly hatched Ephestia larvae.

G. Non-Physical Factors Which Affect Host-Parasite Balance

If the parasites and caterpillars are kept in cylindrical jars or Petri dishes the parasite population can become equal to that of the host at temperatures above 15°C. A reserve supply of host caterpillars must be maintained in order to keep the parasites in food. However, if the same ratio of hosts and caterpillars (100:10 or 10:1) is put in a card-board box with square corners the parasite either does not exterminate the host or requires a longer time to do so than would be expected from its reproductive potential. The corners of the box enable the host caterpillars to escape from the parasite. The edges seem to furnish attachments for the webbing which the caterpillar spins. Once a caterpillar is spun up, it is very difficult for the parasite to reach it.

Another factor which affects host-parasite balance is the strong positive photo-tropism of the Microbracon adult and the strong negative photo-tropism

of the Ephestia larvae. No detailed study was made of the light reactions of the Mediterranean flour moth and its parasite but it was observed during the course of rearing that Microbracon adults flew directly toward the light source upon emerging. Transferring and feeding of both host and parasite were done on a table facing a window. During transferring operations it was observed that the host larvae tended to crawl away from the window, and that parasite adults tended to fly directly to it. The Microbracon female is attracted to the host caterpillar by a chemical stimulus, probably of olfactory nature. Thus, if light is admitted to a breeding chamber in which there are Ephestia larvae, there are two conflicting stimuli for the Microbracon adults.

As has been stated before in the discussion of the literature there are two kinds of *Ephestia kühniella* larvae. One of these is pink, the other yellowish. Both colors occur in each strain used. Although the pink larvae can pupate to produce apparently normal moths, they are not as good food for the Microbracon larvae as are the yellow larvae. When parasites feed on the pink larvae many stunted individuals are produced. These stunted Microbracon lay fewer eggs than the normal and in some cases are sterile.

Although the exact way in which the color of the larvae of Ephestia is inherited is not known, color differences appear to be genetic. Complicating factors in determining the genetics of color are changes of color with advancing age of the larvae, and the effect of certain environments. First instar larvae are pink regardless of what color they will be when full grown. Later the pink coloration disappears and some of the larvae become white or yellowish. Others apparently retain the pink coloration, although it is possible that the pigment of the young larvae is not identical with that of the full grown larvae, even when they have the same general color. Moths reared from pink larvae mated to moths from pink larvae had as offspring both pink and white larvae. Moths reared from white larvae mated to moths reared from white larvae gave white larvae only. Larvae reared from a cross between individuals from pink larvae and those from white or yellowish larvae gave at times both pink and white larvae; at times pink larvae only.

The host larvae is susceptible to both bacterial and protozoan diseases. One of the bacterial diseases not only killed the host but also the parasite larva feeding upon it. Generally if the host becomes diseased the parasite is also infected or may become undernourished. It usually dies with the diseased host.

One factor which may be very important in determining whether the host can survive the parasite is the presence of "Microbracon immune" host larvae. The degree of Microbracon immunity to Ephestia larvae was determined by allowing 10 Microbracons access to 100 Ephestia larvae. At the end of the first day those Ephestia not stung were exposed to fresh Microbracon. If

they were not stung by the second day they were exposed a third, and so on, until the only larvae remaining were those which Microbracon did not attack, although in some cases there were no other larvae available. About one larva in 3,000 appeared to be absolutely immune to Microbracon attack. In some cultures certain larvae are never attacked, although others may be stung repeatedly. Thus there seems to be both relative and absolute immunity to Microbracon. It is not known why some larvae are attacked and others not. There is certainly no striking morphological difference between those attacked and those not, but there may be some difference in odor, since freshly washed Ephestia larvae are seldom attacked.

Freshly hatched Ephestia larvae are never attacked if there are full grown larvae available. In a population containing host larvae of all ages, the youngest larvae would be immune. In some of the calculations given it is assumed that the Microbracon adults are set on hosts at the beginning of the larval stage. In reality the parasites would not be able to develop if placed on newly hatched Ephestia larvae. However, in all cases above 15°C, the larval period of the host was long enough to allow the parasites to reproduce twice and still give time for the host larvae to grow to suitable size to be preyed upon. At temperatures above 15°C the parasite is able to exterminate the host when host and parasite are placed together (in 10:1 ratio) in a closed cylindrical container.

Thus, the calculations are not misleading if they be regarded as expressing the potentialities of the host-parasite balance. In these experiments there is no allowance made for overlapping of generations of either host or parasite. In a later paper the problem of overlapping will be discussed.

H. Host-Parasite Balance When the Parasitic Efficiency of the Parasite Is Less Than Its Reproductive Potential

The number of generations that a parasite requires to control a host, if the parasitic efficiency per generation of parasite is less than the reproductive potential, may be found by substituting the expression "parasitic efficiency" for that of reproductive rate of the parasite in the Thompson (1922) formula, as modified by Chapman (1925).

Thus, if reproductive potential per generation equals parasitic efficiency per generation, the number of generations of parasite required to destroy the host is

$$\frac{\log \Theta}{\log \beta}, \text{ where } \Theta = \frac{w}{z} \text{ or } \frac{\text{reproductive potential of host}}{\text{reproductive potential of parasite}}$$

$$\text{and } \beta = \frac{zp}{n(z-w) + zp},$$

where z = reproductive potential of parasite

p = the original number of parasites

n = the original number of hosts
w = the reproductive potential of the parasite

If the parasitic efficiency, or the number of host killed per generation of parasite, is less than the reproductive potential of the parasite, the number of parasite generations, k, required for the parasite population to equal that of the host may be expressed by the formula

$$k = \frac{Log\ \Theta}{Log\ \beta}, \text{ where } \Theta = \frac{w}{\frac{z}{m}} = \frac{\text{reproductive potential of host}}{\frac{\text{reproductive potential of parasite}}{\text{number of eggs laid per host}}}$$

$$\text{and } \beta = \frac{\frac{zp}{m}}{n(\frac{z}{m}-w) + \frac{zp}{m}}$$

The new factor, m, introduced into the equation represents the average number of eggs laid on each host by a parasite. Since parasitic efficiency can be calculated if the data on reproductive rate are known the expression $\frac{z}{m}$ may be represented by one letter, ϵ, to designate parasitic efficiency. Then the equation becomes:

$$k = \frac{log\ \Theta}{log\ \beta}, \text{ where } \Theta = \frac{w}{\epsilon}, \beta = \frac{\epsilon p}{n(\epsilon - w) + \epsilon p}.$$

If w is greater than ϵ, the parasite cannot control the host. If ϵ is greater than w, unless some factor enters other than reproductive rates or number of eggs laid on a host by one parasite, the parasite population will exceed that of the host.

In the example of host-parasite balance studied in this paper, host and parasite do not reproduce synchronously. The longer cycle of the host keeps the host number constant over a period in which the parasite numbers are increasing. Thus, let

n = the initial number of hosts,
p = the initial number of parasites
z = the reproductive power of the parasite
m = the number of parasite eggs per host.

At the end of the first generation of parasite, the number of hosts is $\frac{zp}{n\text{-}m}$. At the end of the second generation of parasites the number of hosts is $n - (\frac{zp}{m} - \frac{z^2p}{m})$. At the end of the third generation of parasites the number of hosts is $n - \frac{(zp - z^2p - z^3p)}{m\ m\ m}$.

When the equation $n\left(\frac{zp}{m} - z\frac{a+1}{m}p - z\frac{a+2}{m}p - \ldots . z\frac{a+k}{m}\right) = 0$, the parasite has destroyed the host. Generally the number inside the parenthesis is much larger than the original host number at the time when the parasite destroys the host.

Practically the mathematical growth of the expression inside the parenthesis means that hosts must be added to the original parasite-host population if the parasites are to have enough food.

I. Extreme Survival Temperatures of Host and Parasite

Even if the reproductive rate of the parasite is greater than that of the host at medial temperatures, it is possible that the host by its ability to withstand temperature extremes might survive when the parasite would die. The determination of survival temperatures is by no means easy, since the survival temperature is affected by the past history of the insect, by the genetic strain, and by the humidity. However, there exist some reliable data for both Ephestia and its parasite. Goodwin (1914) found that the larvae died at 43-46°C, and the eggs at 47.5 to 48°C. Dean (1913) found 46.6°C fatal to adults, 47.77°C fatal to larvae, and 49.44°C to eggs. Both workers found the eggs more resistant to high temperatures than the active larvae. Dieuzeide (1926), who studied Algerian Ephestia, found that all stages were killed by temperatures from 47.44-51.66°C. Dieuzeide wished to make sure that all individuals were killed, since he was advocating heat as a control measure. Therefore the temperatures actually required to kill might be somewhat lower. He also found at the other extreme that temperatures as low as -2.66°C and -1.11°C would kill all stages. A temperature of -2.66°C is rather high for a fatal limit, but it must be remembered that Dieuzeide worked with a strain from a warm climate. The present writer found that larvae of Ephestia from Minnesota were killed by -15°C. Some few determinations made from Pennsylvania larvae showed a death point of from -7°C to -12°C. The determinations by Dieuzeide were not only made on individuals from a warm climate, but also involved the quantity factor of low temperature, since the larvae were exposed to the low temperature for some time. The determinations by the author were made in such a way as to exclude as far as possible the quantity factor of low temperature. A span of from -15°C to near +50°C would represent quite fairly the temperatures at which Ephestia can live.

The upper fatal limit of *Microbracon hebetor* adults depends upon the temperature at which they have been reared. Since light colored adults develop at high temperatures and black ones at low temperatures, it may be said in general that light adults are able to withstand a higher temperature than dark adults. Pure yellow adults reared at 36°C die at a temperature of 65°C.

Black adults reared at 16.5°C are killed at 61°C. The external temperature was measured by a thermocouple when the animals were in the sunlight. The internal temperature of the black adult measured at the same time by a thermocouple was 63°C and that of the light adult the same. It may thus be said that an internal temperature of 63°C will kill adults of either color. The fatal temperature limit for the larvae was 38°C, even for those reared at 36°C. Eggs and pupae were killed at 40°C. Adults reared at low temperatures were able to withstand temperatures as low as -12°C; those reared at high temperatures died at -7°C to -8°C. Feeding larvae were the most sensitive to cold, since they were killed at temperatures but little below 0°C, namely -1°C or -2°C. Pupae and eggs were not killed until -14°C was reached. The total span of environmental temperature over which some stage of the parasite can live is about -12°C to + 65°C. Since the larvae are killed at 38°C and the larvae of the flour moth not till 43-46°C it is possible that extreme high temperature might damage the parasite more than the host. The weak point in the parasite life cycle is the tender larval stage.

J. Cold-Hardiness of Host and Parasite to the Quantity Factor of Low Temperature

Both host and parasite are extremely cold-hardy to the quantity factor of low temperature. The larvae of Ephestia may be kept in ice boxes for months without injury. Whiting, 1921, has used this property of cold-hardiness to the quantity factor of low temperature in his work. He also successfully stored Microbracon in ice boxes if there was no food for it. Both host and parasite are able to withstand temperatures from 0 to + 4°C for periods of 4-6 months without showing injury. Hase (1927) reported that there was retardation of development proportional to the length of time the eggs of *E. kühniella* had been kept at low temperatures. This retardation was considered as a sign of injury by Janisch (1925). However, the fact remains that both host and parasite have the capacity of surviving long periods of exposure to temperatures below their threshold of development.

V. SUMMARY

1. There are at least two strains of the Mediterranean flour moth. One of these passes through a complete life cycle in 35-37 days at 32°C; the other strain requires 62-65 days at the same temperature. The type of developmental curve of these two strains plotted against temperature is similar. The threshold temperature of development of the slow strain is + 8°C; of the fast one, + 10°C.

2. There are several strains of *Microbracon hebetor* Say, a brachonid parasite of *E. kühniella*. Only one genetic strain has been studied in detail. This stock was characterized by great healthiness, freedom from dwarfs and

abnormal individuals, and by a high reproductive potential except at extreme temperatures; 100% of the eggs laid, hatched. The females were nearly 100% fertile. From 7-8 days was required from egg to emergence of imago at 36°C and from 30-40 days at 15°C.

3. The parasite, when placed in a culture of host larvae in 100:10 ratio, is able to destroy the host at all temperatures above 15°C, if the hosts are so placed that none of them are able to protect themselves by thick webbing or to hide in dark corners.

4. The Mediterranean flour moth is very sensitive to low humidity; the parasite is extraordinarily resistant to drying.

5. The total temperature scale at which the Mediterranean flour moth can live extends from about -15°C to + 50°C. Race and previous history of the individual affect the extremes of temperature which this insect can endure.

6. The total temperature scale of the strain of *Microbracon hebetor* used in these experiments is from -12°C to + 65°C (external temperature). At a given environmental temperature the internal temperature of light colored forms is less than that of dark colored. Yellow adults are produced at high temperatures and black ones at low.

7. Both host and parasite are extremely cold-hardy to the quantity factor of low temperature.

8. A general formula,

$$k = \frac{\log \frac{(w)}{\epsilon}}{\log \frac{(\quad \epsilon p \quad)}{n(\epsilon - w) + \epsilon p}}$$

may be used to find the number of generations required for the parasite to overtake the host, if both host and parasite reproduce synchronously. In the above equation, k = number of generations required for the parasite to exterminate the host, w = reproductive potential of the host, ϵ = the parasitic efficiency of the parasite, or the net number of hosts killed per generation of parasite, and n = initial number of hosts.

9. However, since the host and parasite do not reproduce synchronously, host-parasite balance is determined by two factors in addition to the relative reproductive potentials per generation of host and parasite: (1) by the number of generations of parasite which develop on that stage of the host upon which the parasite feeds; (2) on the length of time the parasite can endure starvation should it chance to emerge when the only stages of the host present are those upon which it cannot feed.

VI. CONCLUSIONS

Under certain conditions of temperature and moisture, the brachonid parasite *Microbracon hebetor* Say, is able to control its host *Ephestia kühniella*

Zeller. The parasite is favored by a shorter life cycle, whereby it can reproduce several times while the host is reproducing once. Since the parasite feeds on the host larva, the relative length of this host stage favors the parasite. The fast strain of host is less susceptible to parasitic attack than the slow one, since the slow one furnishes nourishment for a longer time than does the fast one and thus allows more generations of parasite to develop on it. If the adult parasite emerges when the host is in the egg or the pupal stage the slow strain has the advantage, since the parasite has to starve longer before it obtains food. Considering the life cycle as a whole, the fast strain is less susceptible to parasitic attack. In certain limiting cases, it is possible, however, for the fast strain to be exterminated when the slow one could survive.

The importance of small differences in reproductive rate becomes very great at temperatures where the host-parasite balance is delicate. For example, at 15°C a retardation of a day in the development of the host may mean that the parasite can destroy it. On the other hand, death of even a few parasite larvae may tip the balance in favor of the host.

The possibility of a parasite controlling a host cannot be decided by the end of the first generation. Even a very efficient parasite may not seem to be making much progress at the end of the first generation, but by the end of the second or third generation it may exterminate the host.

Non-physical factors complicate the problem of host-parasite balance. Among these is the ability of the host larva to escape attack. Part of this ability is due to the fact that the larva is negatively phototropic and the parasite adult positively phototropic. Occasionally there is a "Microbracon immune" larva which is never stung by the parasite wasp. Such occasional immune larvae might be able to perpetuate the race.

The association of *Microbracon hebetor* Say with its host, *Ephestia kühniella*, appears to be of recent origin. The lack of synchrony in the life cycles and the opposite responses to light and moisture point to an imperfect adaptation of host and parasite.

The experimental data on the effect of temperature sheds some light on the possible original home climate of both host and parasite. Since the host is extremely sensitive to low humidity and all stages of it cannot develop above 34.4°C it could not have originated in a hot, dry climate. A special micro-climate such as that in wild bee's nests, could of course enable it to live where the general climate was unfavorable. The parasite is, however, adapted to a hot, dry, almost desert, climate. The only stage which is not resistant to drying is the feeding larva. All the other stages can be reared over $CaCl_2$. These include the egg, spun larva, pre-pupa, pupa, and adult or more than three-fourths of the total life cycle.

BIBLIOGRAPHY

Ashmead, W. H. 1889. Descriptions of new Braconidae in the collection of the U. S. National Museum. Proc. U. S. Nat. Mus. **11:** 611-671.

Back, E. A. 1920. Insect control in flour mills. U. S. Dept. Agri. Bull. 872. 40 pp.

Baker, C. F. 1895. Two new Apanteles. Ent. News **6:** 201-202.

Barnes, H. F. 1930. On some factors governing the emergence of gall midges: Cecidomyidae: Diptera. Proc. Zoöl. Soc. London **1930:** 381-393.

Berliner, E. 1915. Über die Schlaffsucht der Mehlmottenraupe. (*Ephestia kühniella* Zell.) und ihren Erreger *Bacillus thuringensis* n.sp. Zeitschr. f. angew. Ent. **2:** 25-26.

Blunk, H. 1929. Die Erforschung epidemischer Pflanzenkrankheiten auf Grund der Arbeiten über die Rübenfliege. Zeitschr. f. Pflanzenkrankh. **39:** 1-28.

Blunk, H., H. Bremer, und O. Kaufmann. 1928. Untersuchungen zur Lebensgeschichte und Bekämpfung der Rübenfliege (*Pegomyia hyoscini* Panz.) Arb. biol. Reichsanstalt. f. Land u. Forstwirtsch. **16:** 423-573.

Bremer, H. 1926. Über die tageszeitliche Konstanz im Schlüpftermine der Imagines einiger Insekten und ihre experimentelle Beeinflussbarkeit. Zeitschr. f. wissensch. Insektbiol. **21:** 209-216.

1928. Geschichte des Rübenfliegenbefalles in nördlichen Vorpommern und auf Rügen im Jahre 1925. Arb. biol. Reichsanstalt f. Land. u. Forstwirtsch. **16:** 448-468.

Bridgeman, J. B. 1889. Notes on *Ephestia kühniella.* Insect Life **2:** 260.

Brindley, T. A. 1930. The growth and development of *Ephestia kühniella* Zeller (Lepidoptera) and *Tribolium confusum* Duval (Coleoptera) under controlled conditions of temperature and relative humidity. Ann. Ent. Soc. America. **23:** 741-757.

Buchwald, J. und E. Berliner. 1910. *Habrobracon hebetor* Say. Ein Bundesgenosse im Kampf gegen die Mehlmotte. Zeitschr. f. das ges. Getreidewesen **2:** 1-4.

Burkhardt, F. 1919. Zur Biologie der Mehlmotte. (*Ephestia kühniella* Zeller. Zeitschr. f. angew. Entom. **6:** 25-60.

Candura, G. S. 1928. Contributo alla conoscenza della tignola grigia delle proviste alimentari *(Ephestia kuehniella)* e del suo parasita *Nemeritis canescens* Gravenhorst. Boll. Lab. Zoöl. Portici. **21:** 149-214.

Chapman, R. N. 1925. Animal Ecology with especial reference to insects. Burgess-Brook. Inc. Minneapolis. 370 pp. See pp. 158-161.

1928. The quantitative analysis of environmental factors. Ecology **9:** 111-122.

Chittenden, F. H. 1909. Control of the Mediterranean flour moth by hydrocyanic-acid gas fumigation. U. S. Dept. Agri. Bur. Ent. Cir. 112. 43 pp.

Cook, W. C. 1927. Some effects of alternating temperatures on the growth and metabolism of cutworm larvae. Jour. Econ. Ent. **20:** 769-782.

Cushman, R. A. 1914. A revision of the North American species of the Braconid genus Habrobracon Johnson (Ashmead). Proc. Ent. Soc. Washington. **16:** 99-108.

1922. The identity of *Habrobracon brevicornis* (Wesmael). (*Hym.* Braconidae.) Proc. Ent. Soc. Washington. **24:** 241-242.

Danysz, J. 1893. *Ephestia kuehniella,* parasite de Blés, des farines, et des biscuits. Histoire naturelles du parasite et moyons de la détruire. Mémoires du laboratoire de Parasitologie Végétale de la Bourse de commerce. Paris. **1:** Reviewed in Insect Life **5:** 290-291.

Daviault, L. 1930. Notes biologiques sur *Nemeritis canescens* Grav. et sur la morphologie de ses divers stades. Rev. Path. vég. ent. agric. **17:** 82-93.

Dean, G. A. 1913. Mill and stored-grain insects. Kansas Agri. Expt. Sta. Bull. **189:** 223-228.

Diamond, V. R. 1929. The biology of *Nemeritis canescens*, a parasite of the Mediterranean flour moth. Ann. Rept. Ent. Soc. Ontario. **60:** 84-89.

Dieuzeide, R. 1926. La papillon gris de la farine. *Ephestia kuehniella* (Zeller). Rev. Zoöl. agri. e appl. **25:** 1725.

Doten, S. B. 1911. Concerning the relation of food to reproductive activity and longevity in certain hymenopterous parasites. Nevada Agri. Exp. Sta. Tech. Bull. **78.** 30 pp.

van Emden, F. 1929. Ueber die Rolle der Feuchtigkeit im Leben der Speicherschädlinge. Anz. f. Schädlingsk. **5:** 58-60.

Escherich, K. und M. Miyajima. 1911. Studien über die Wipfelkrankheit der Nonne. Naturw. Zeitschr. Forst. Landw. **9:** 381-402.

1911. Studien über die Wipfelkrankheit der Nonne. Biol. Zentralblatt **32:** 111-119.

Evans, J. W. 1930. The control of the codling moth in Australia. Notes on the possible utilization of Trichogramma (Hym. Chalcidoidea.) Jour. Council Sci. Indian. Res. **3:** 106-116.

Fölsch, W. 1926. Ueber die Mehlmotte, *Ephestia kühniella*. Zeller. Anz. f. Schädlingsk. **2:** 98-99.

Friend, R. B. 1927. The biology of the birch-leaf skeletonizer *Buccalatrix canadensisella*. Connecticut Agri. Exp. Sta. Bull. **288:** 395-486.

Glenn, P. A. 1909. The influence of climate upon the green bug and its parasites. Kansas Univ. Sci. Bull. **9:** 165-200.

Goodwin, W. H. 1914. Some factors affecting results in the use of high temperature for the control of insects injuring cereal products. Jour. Econ. Ent. **7:** 313-322.

Hase, A. 1922. Biologie der Schlupfwespe, *Habrobracon brevicornis* Wesmael. (Braconidae) zugleich ein Beitrag zur Frage der biologischen Bekämpfung von Schädinsekten. Arb. biol. Reichsanstalt. f. Land. u. Forstwirtsch. **11:** 95-186.

1925. Weitere Versuche zur Frage der biologischen Bekämpfung von Mehlmotten mit Helfe von Schlupfwespen. Arb. biol. Reichsanstalt. f. Land. u. Forstwirtsch. **19:** 163-169.

1927. Über Temperaturversuche mit den Eiern der Mehlmotte (*Ephestia kühniella* Zell.). Arb. biol. Reichsanstalt. f. Land. u. Forstwirtsch. **15:** 109-133.

Headlee, T. J. 1914. Some data on the effect of temperature and moisture on the rate of insect metabolism. Journ. Econ. Ent. **7:** 413-417.

Hefley, H. M. 1928. Differential effects of constant humidities on *Protoparce quinquemaculatus* Haworth, and its parasite *Winthemia quadripustulata*. Fabricus. Jour. Econ. Ent. **21:** 213-221.

Herfs, A. 1926. Oekologische Untersuchungen an *Pediculoides ventricosus* (Neup.) Berl. Zoölogica **28:** 1-68.

Hunter, S. J. 1909. The Green Bug and its Enemies. Kansas Univ. Bull. **9:** 1-161.

Janisch, E. 1925. Über die Temperatur abhängigkeit biologischer Vorgänge und ihre kurvenmässige Analyse. Pflüger's Arch. ges. Physiol. **209:** 414-436.

1927. Das Exponentialgesetz als Grundlage einer vergleichenden Biologie. Abhandl. Theorie organ. Entwickl. Hft. **2:** 383 pp.

1930. Experimentelle Untersuchungen über die Wirkung der Umweltfaktoren auf Insekten i. Die Massenvehmehrung der baumwolleule. *Prodenia littoralis*. Zeitschr. f. wiss. Biol. Abteil. A. Zeitschr. f. Morph. u. Ökol Tiere **17:** 339-416.

Johnson, W. G. 1894. A new parasite of the Mediterranean flour moth *(Ephestia kühniella)*. Ent. News **6:** 324-325.

1895. Miscellaneous Entomological Notes. Bull. U. S. Dept. Agri. **20:** 62-68.

Kalendar, E. 1872. Untersuchungen über beschleunigte Entwicklung überwinternden Schmetterlings-Puppen mit Berücksichtigung der Lebens-und Verwandlungs- art dieser Puppen-Inaugural Dissertation. Univ. Rostock. 30 pp.

Kaufmann, O. 1925. Beobachtungen und Versuche zur Frage der Überwinterung und Parasitierung von Ölfruchtschädlingen aus der Gattungen *Meligheles, Phyllotreta, Psylloides*, und *Centorrhynchus*. Arb. biol. Reichsanstalt. f. Land. u. Forstwirtsch. **12:** 109-169.

Kirby, W. F. 1884. (Note without title) Trans. Ent. Soc. London. **1884**. In Proceedings, p. xxxi.

Klein, S. T. 1887. (Note without title) Trans. Ent. Soc. London. **1887:** LII-LIV.

Kornauth, K. 1917. Bericht über die Tätigkeit der k- k, landw-bakter-und Pflanzenschutz station in Wien im Jahre 1916. Zeitschr. f. landw. Versuchswesen in Oesterreich. **20:** 288-314.

Krogh, A. 1914. On the rate of development and CO_2 production of chrysalids of *Tenebrio molitor* at different temperatures. Zeitschr. f. allgem. Physiol. **16:** 178-190.

Krüger, P. 1920. Beobachtungen am Mehlmottenparasiten *Nemeritis canescens* Gravenhorst; zugleich ein Beitrag zur Kenntnis der äusseren Anatomie der Ichneumoniden. Zeitschr. f. angew. Ent. **7:** 58-67.

Lebedev, A. G. 1929. On the question of the geographical origin of the flour moth, *Ephestia kühniella* Zell. (In Russian.) Plant Protection (Leningrad) **6:** 45-51.

Marshall, T. A. 1885. Monograph of the British Braconidae. Part 1. Trans. Ent. Soc. London **1885:** 1-280.

Mattes, O. 1927. Parasitäre Krankheiten der Mehlmottenlarven und Versuche über ihre Verwendbarkeit als biologisches Bekämpfungsmittel. Zugleich ein Beitrag zur Zytologie der Bakterien. Sitz-Ber. Ges. Beförd. gesam. Naturw. Marburg. **62:** 381-417.

1928. Über den Entwicklungsgang der Microsporidie *Thelohania ephestiae* und die von ihr hervorgerufenen Krankheitserscheinungen. Zeitschr. f. wisschensch. Zoöl. **132:** 526-608.

Muesebeck, C. F. W. 1925. A revision of the parasitic wasps of the genus Microbracon occurring in America north of Mexico. Proc. U. S. Nat. Mus. **67** (article 8): 1-85.

1926. Descriptions of new reared parasitic Hymenoptera and some notes of synonomy. Proc. U. S. Nat. Mus. **69:** (article 7): 1-18.

Muir, F. 1913. Presidential address. Proc. Hawaiian Ent. Soc. **3:** 28-32.

Ormerod, E. A. 1889. The new flour moth in England. (In letter to C. V. Riley) Insect Life. **1:** 314-315.

Payne, N. M. 1927. Two factors of heat energy involved in insect cold hardiness. Ecology **8:** 194-196.

Pearl, R. 1925. The biology of population growth. Alfred A. Knopf. New York. 260 pp.

Pearl, R. and S. L. Parker. 1922. Experimental studies on the duration of life. II. Hereditary differences in duration of life in line-bred strains of Drosophila. Amer. Nat. **56:** 174-187.

1922. Experimental studies on the duration of life. V. On the influence of certain environmental factors on duration of life in Drosophila. Amer. Nat. **56:** 385-405.

Pemberton, C. E. and H. F. Willard. 1918. Interrelations of fruit-fly parasites in Hawaii. Jour. Agri. Res. **12:** 285-295.

Freudhomme de Borre 1884. (Note without title) Compt. Rend. Soc. Ent. Belgique. **28:** ccxxxvi-ccxxxvii.

Prochnow, O. 1907. Die Temperaturkurve der Entwicklungsgeschwindigkeit für Pflanzen und poikilotherme Tiere. Ent. Zeitschr. Guben. **20:** 313-314.
1908. Die Abhängigkeit der Entwicklungs-und Reaktions- geschwindigkeit bei Pflanzen und poikilothermen Tieren von der Temperatur. Inagural-Dissertation. Universität-Berlin. 39 pp.

Richardson, C. H. 1926. A physiological study of the growth of the Mediterranean flour moth (*Ephestia kühniella*. Zell.) Jour. Agri. Res. **32:** 895-929.

Riley, C. V. 1889. The so-called Mediterranean flour moth (*Ephestia kühniella* Zeller). Insect Life. **2:** 166-171.

Riley, C. V. and L. O. Howard. 1895. A new grain moth parasite. [In notes from correspondence.] Insect Life. **7:** 428.

Ruzička, J. 1924. Die neuesten Erfahrungen über die Nonne in Böhmen. Centralblatt. f. das. ges. Forstw. **50:** 33-67 ; 313-335.
1925. Einige Bemerkungen über die Nonnenbekämpfung auf biologischen Wege. Forstwiss. Zentralbl. **47:** 537-538.

Sanderson, E. D. 1910. The relation of temperature to the growth of insects. Jour. Econ. Ent. **3:** 113-140.

Say, T. 1836. Descriptions of new species of North American Hymenoptera and observations on some already described. Boston Jour. Nat. Hist. **1:** 210-305.

Shelford, V. E. 1926. The relation of abundance of parasites to weather conditions. Jour. Econ. Ent. **19:** 283-289.

Schlottke, E. 1926. Über die Varabilität der schwarzen Pigmentierung und ihre Beeinflussbarkeit durch Temperaturen bei *Habrobracon juglandis*. Ashmead. Zeitschr f. wissensch. Biol. Abteil. C. Zeitschr. f. vergleich Physiol. **3:** 692-736.

Schulze, H. 1926. Ueber die Fruchtbarkeit der Schlupfwespe *Trichogramma evanescens* Westwood. Zeitschr. f. wiss. Biol. Abteil A. Zeitschr. f. Morph. u. Ökol. Tiere. **6:** 553-585.

Snellen, P. C. T. 1881. *Ephestia kühniella* Zell. Tijdschr. v. Ent. **24:** xx-xxi.

Smits van Burgst, C. A. L. 1921. Parasieten van het Meelmotje (*Ephestia kühniella*, Zeller) Tijdschr. Plantenziekten. Wageningen. **27:** 77-79.

Thompson, W. R. 1922. Étude de quelques cas simples de parasitisme cyclique chez les insectes entomophages. Compt. Rend. Acad. Sci. (Paris) **174:** 1647-1649.
1922. Théorie de l'action des parasites entomophages. Accroisment de la proportion d'hôtes parasites dans le parasitisme cyclique. Compt. Rend. Acad. Sci. (Paris) **175:** 65-68.

Viereck, H. L. 1911. Descriptions of six new genera and thirty-one new species of Ichneumon flies. Proc. U. S. Nat. Mus. **40:** 170-196.

Voinovskaya-Kriger, T. 1929. On the biology of *Nemeritis canescens* Grav. (Hymenoptera Ichneumonidae)—Parasite of *Ephestia kühniella* Zell. (In Russian with German summary.) Rept. Bur. appl. Ent. (Leningrad) **3:** 24-35.

Voukassovitch, P. 1929. Observations biologiques sur un Lépidoptère (*Ephestia kuchniella* Zell.) Compt. Rend. Soc. Biol. **100:** 62-64.
1929. Nouvelles observations sur *Ephestia kuchniella* Zell. Compt. Rend. Soc. Biol. **100:** 64-67.

Wadsworth, R. V. 1919. Notes on the life-history of *Ephestia kühniella*. Ann. appl. Biol. **6:** 203-206.

Walley, G. S. 1929. Descriptions of new Canadian parasitic Hymenoptera. Can. Ent. **61:** 190-194.

Washburn, F. L. 1904. The Mediterranean flour moth, *Ephestia kühniella*, Zell. Minnesota State Entomologists' Ninth Report. Pp. 17-40.

Webster, F. M. and W. J. Phillips. 1912. The spring grain-aphis or "green bug." Bull. Bur. Ent. U. S. Dept. Agri. 110. 153 pp.

Welter, A. 1895. Die tiefen Temperaturen. Crefeld. 86 pp.

White, G. F. 1927. A protozoan and a bacterial disease of *Ephestia kühniella* Zell. Proc. Ent. Soc. Washington. **29:** 147-148.

Whiting, P. W. 1921. Rearing meal moths and parasitic wasps for experimental purposes. Jour. Heredity. **12:** 255-261.

1921. Heredity in wasps. A study in a parthenogenetic insect, the parasitic wasp, Habrobracon. Jour. Heredity. **12:** 262-266.

Zacher, F. 1922. Eingeschleppte Vorrätsschädlinge gefahren für unser Wirtschaftsleben. Die Umschau [Frankfort-am-Main] **26:** no. 5. 4 pp.

1928. Versuche über den Einfluss der Verpackung auf den schädlingsbefall. Mitt. ges. Vorrätschäd. **4:** 2-5.

Zeller, P. C. 1881. Lepidopterologische Bemerkungen. Stettiner Ent. Zeitung **40:** 462-471. See pp. 466-471.

THE ROLE OF COPPER IN THE SETTING, METAMORPHOSIS, AND DISTRIBUTION OF THE AMERICAN OYSTER, *OSTREA VIRGINICA*

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THE ROLE OF COPPER IN THE SETTING, METAMORPHOSIS, AND DISTRIBUTION OF THE AMERICAN OYSTER, *OSTREA VIRGINICA*

INTRODUCTION

Importance of the Study

The most important and critical period in the life history of the oyster is that during which the fully developed larva cements itself to some clean submerged surface such as old shells or stones and then undergoes metamorphosis into a spat and adult oyster. Various terms such as setting, striking, spatting, catching, and fixation are used in different oyster regions to designate this process of attachment. Since 1665 studies have been made of the oyster and more written on the history of its development than any other invertebrate and yet very little has been observed or recorded of its behavior during this important setting period. In his excellent report on the Canadian oyster, Stafford (1913) states: "Spatting is the all important event. The value of the oyster harvest does not depend upon the number of eggs spawned, nor upon the number of larvae in the water but upon the number of successful spat."

A study of the location of natural oyster beds reveals at once the fact that they are found only in coastal areas where salt water of the ocean is considerably diluted by drainage of fresh water from the land. The ideal conditions for oyster reproduction, setting, and growth are found primarily in the river mouths, harbors, and bays where water conditions are exceedingly complex from a physical and chemical standpoint. In each particular locality the horizontal and vertical distribution of oysters is relatively the same from year to year. Setting occurs in rather definite zones and is of greatest intensity at certain tidal levels. It is evident, therefore, that attachment of the larva is not a haphazard process but is a definite biological reaction in response to some environmental stimulus. Certain areas are particularly valuable for setting and seed oyster production because the larvae attach there in greater abundance. Consequently the present studies were undertaken with a view to increasing knowledge of the setting of the larva and to determine the principal environmental factors involved in this process.

These studies have been carried out for the past three years at the Bureau of Fisheries field station at Milford, Connecticut and at the Zoölogical Laboratory of the University of Pennsylvania. The author is deeply indebted to Dr. P. S. Galtsoff of the U. S. Bureau of Fisheries and Dr. C. E. McClung of the University of Pennsylvania for advice and assistance during the course of the work and for criticism of the manuscript.

PLAN AND PURPOSE OF THE INVESTIGATION

The collection of seed oysters by artificial methods is a most important phase of modern oyster culture. In order to provide the oyster larvae with

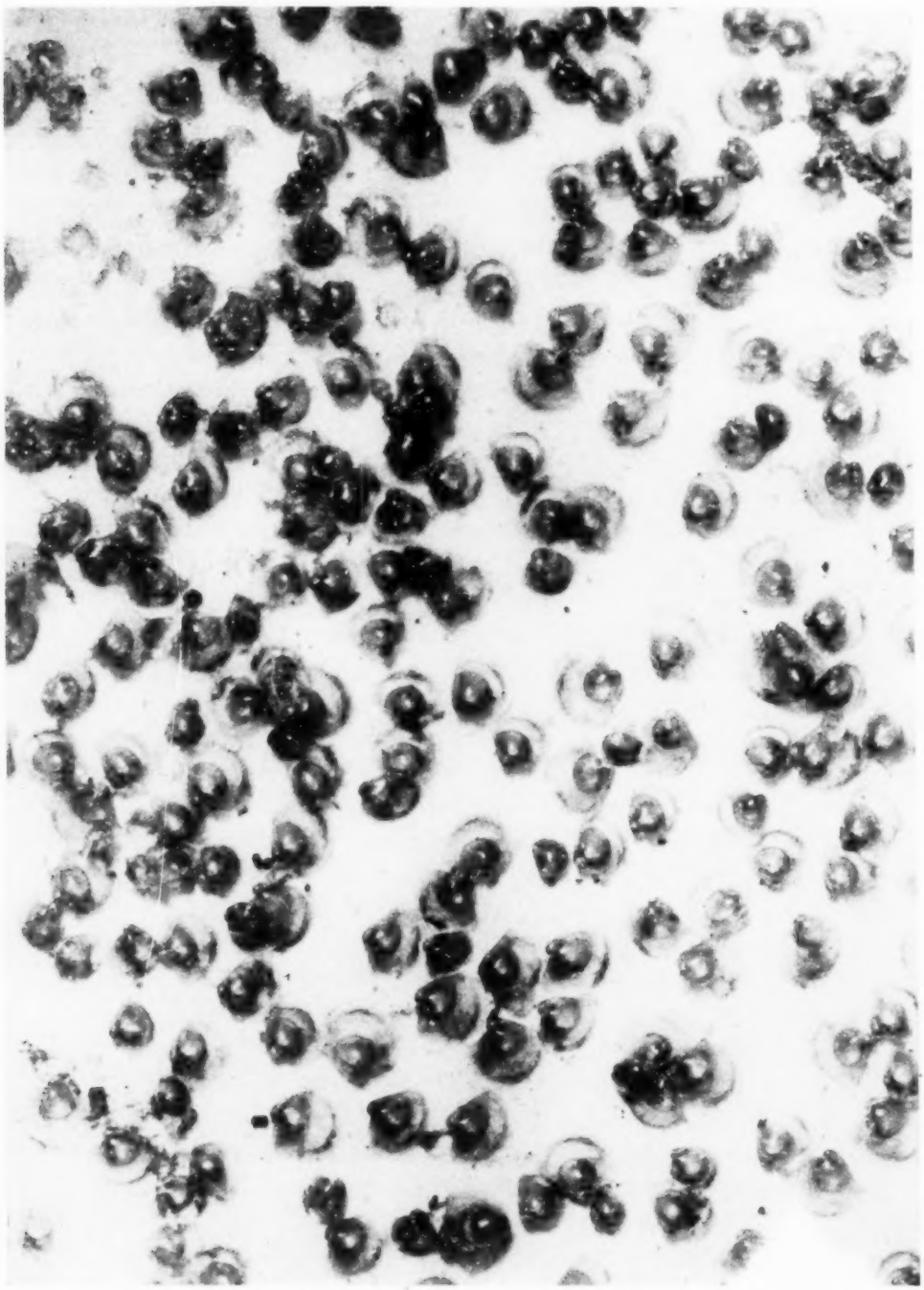

FIG. 1. Small portion of old shell covered with intensive set of oyster spat, 1 to 2 days old.

a suitable surface for attachment, oyster culturists plant old shells, gravel, etc., on the areas that are most favorable for setting and the collection of seed oysters. These operations have gradually become more intensive and costly and meet with varying success from year to year according to seasonal conditions as shown by Prytherch (1929) and also according to the particular area, zone, or locality in which the set collecting material is planted.

Certain areas are of almost inestimable value in oyster farming because setting of the larvae occurs there frequently and in considerable intensity as shown in Figure 1 while adjacent bottoms under apparently similar conditions are for some reason, worthless for this purpose. For example, in Long Island Sound, from the mouth of the Housatonic River to the westward for a distance of several miles, is the greatest seed oyster producing area in Connecticut while to the eastward the bottom is of little value for

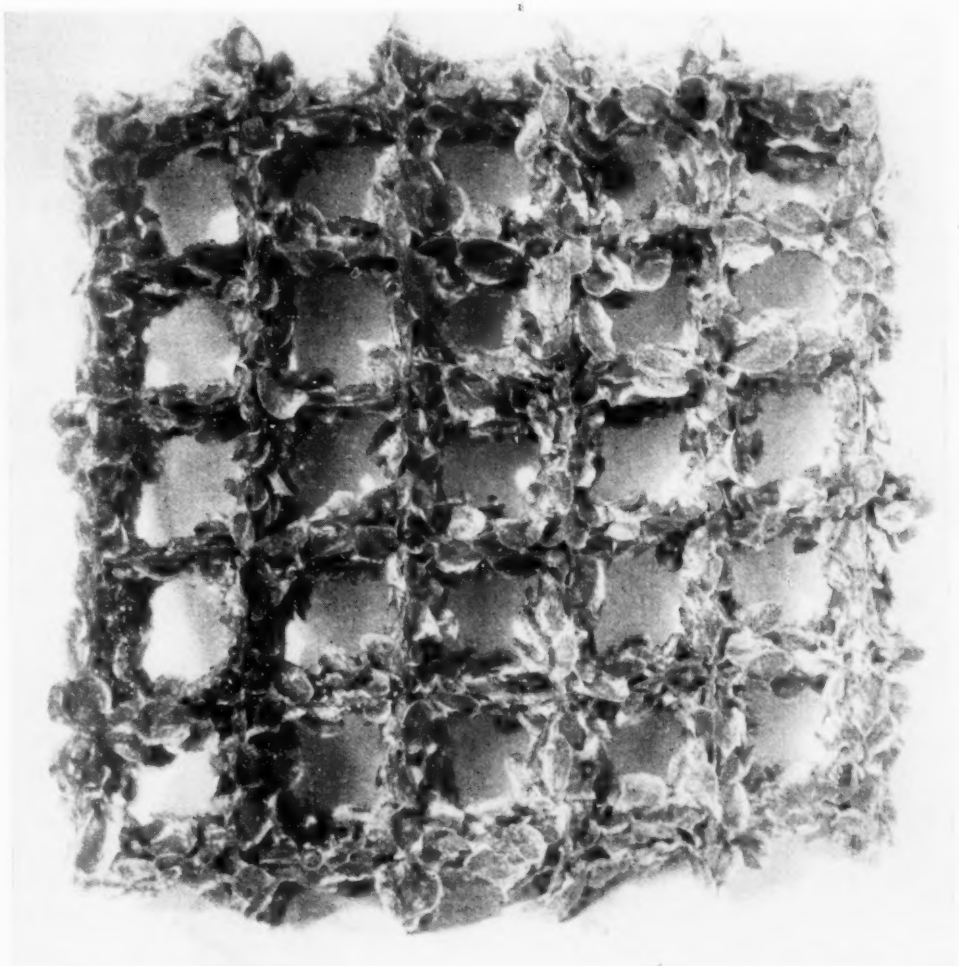

FIG. 2. Heavy set of oysters, 2 months old, on partition collector planted at low water level, Milford Harbor, Conn., 1930.

this purpose. In addition to such horizontal variations in the intensity of setting we also have marked vertical differences (Prytherch 1929; Galtsoff, Prytherch and McMillin 1930). This was also demonstrated remarkably well by experiments in Milford Harbor, Conn., during the summer of 1930 in which the new type of seed collector shown in Figure 2 was tested under various conditions. The collectors planted at low water mark were covered with from 3,000 to 12,000 spat while those elevated 2 feet or more above this zone failed entirely. In this harbor 5 acres of tidal flats lying at low water level thus produced roughly over 100,000,000 seed oysters.

In almost every oyster producing region similar interesting and complex phenomena in respect to oyster setting and distribution were observed. It was evident that a special study should be made of the method of attachment of the larval oyster and the chief factors governing this process. The setting period though of relatively short duration in the development of the oyster is of particular importance as at this time its existence as a sedentary organism begins, its future location is selected, and its possibilities for survival are determined by the ability of the larva to obtain a favorable place for attachment.

The plan of this investigation has been to study setting under natural conditions and in the laboratory in order to determine: First, the process by which the attachment of the larvae is accomplished; and second, the more important physical and chemical factors of the environment which initiate and control this reaction. The field studies show that the distribution of oysters and heavy setting areas can be correlated with certain factors such as salinity, river discharge, and current velocity. In order to determine the relative importance and effect of these factors, the full grown larvae were subjected to experimental procedure under controlled laboratory conditions. Their reactions to each particular environmental factor were studied to determine which exerted the greatest influence on attachment and subsequent metamorphosis of the larva.

The final step in the investigation has been a microscopical study of (1) the structure and function of larval organs; (2) the important morphological changes that occur during setting and metamorphosis; and (3) the possible relation of these changes to the principal controlling environmental factors.

METHODS OF STUDY

The larvae were collected from Milford Harbor by means of a 1-meter tow net of No. 10 bolting silk which retains only those which are full grown. The separation of oyster larvae from other marine forms is greatly simplified by washing the plankton sample from the net into a tall straight sided glass jar with funnel-shaped bottom, into the center of which the shelled larvae of various species soon collect. These were siphoned off with a glass

tube, placed in Syracuse glasses, and forms other than oysters removed under the binocular by means of a fine pipette.

In the laboratory experiments larvae were taken either from one of three stock cultures or directly from plankton samples, the results in either case showing no noticeable differences in their reactions to various stimuli. The solutions of various salts were made up in amounts of 100 to 1,000 c.c. and 10 c.c. portions of each concentration placed in Syracuse glasses to which several larvae were added. Observations as to their reactions were made immediately and later at regular intervals.

The larvae are vigorous swimmers, so it was desirable that some means be devised for immobilizing them which would not seriously interfere with their feeding activities or normal behavior. This was accomplished by cementing the shell of the larva to a glass slide, with a thin solution of marine glue. Since such a method may likewise greatly facilitate the study of other microscopic shelled larvae a brief description of the technique is given as follows:

The larvae are removed from solution with a fine pipette, placed to one side of a glass slide and all the excess water drained away with a small camel's hair brush. A drop of marine glue is placed in the center of the slide and allowed to evaporate for a minute or so until a thin sticky film has been formed. The larvae are then picked up with the tip of the brush, gently placed in the glue in the desired position and the slide transferred to seawater about five minutes later after the glue has set. The oyster larvae were cemented down by the left or larger valve and tilted at an angle of 45° so as to allow normal functioning of the velum.

Observations could then be easily made from either side as to their internal anatomy, feeding habits, effect of vital stains, reactions to various stimuli, etc. Oyster larva immobilized in this manner appeared to grow and develop normally and were similar to free swimming forms in their response to stimuli. In making microphotographs or moving pictures of the larva as shown in Figure 3 this method has proved to be extremely helpful for demonstrating the internal anatomy and functions of the organism.

In determining the effect of various physical and chemical factors on setting the following methods were used: Water samples were collected from the harbor at surface and bottom during several complete tidal cycles and analyzed for oxygen content, salinity, hydrogen-ion concentration, and temperature. For the measurement of dissolved oxygen the Winkler method was employed. The salinity of the water was calculated by means of Knudsen's hydrographical tables from specific gravity readings made with Knudsen hydrometers. For the determination of the hydrogen-ion concentration the colorimetric method was employed using cresol red and brom-thymol blue as indicators.

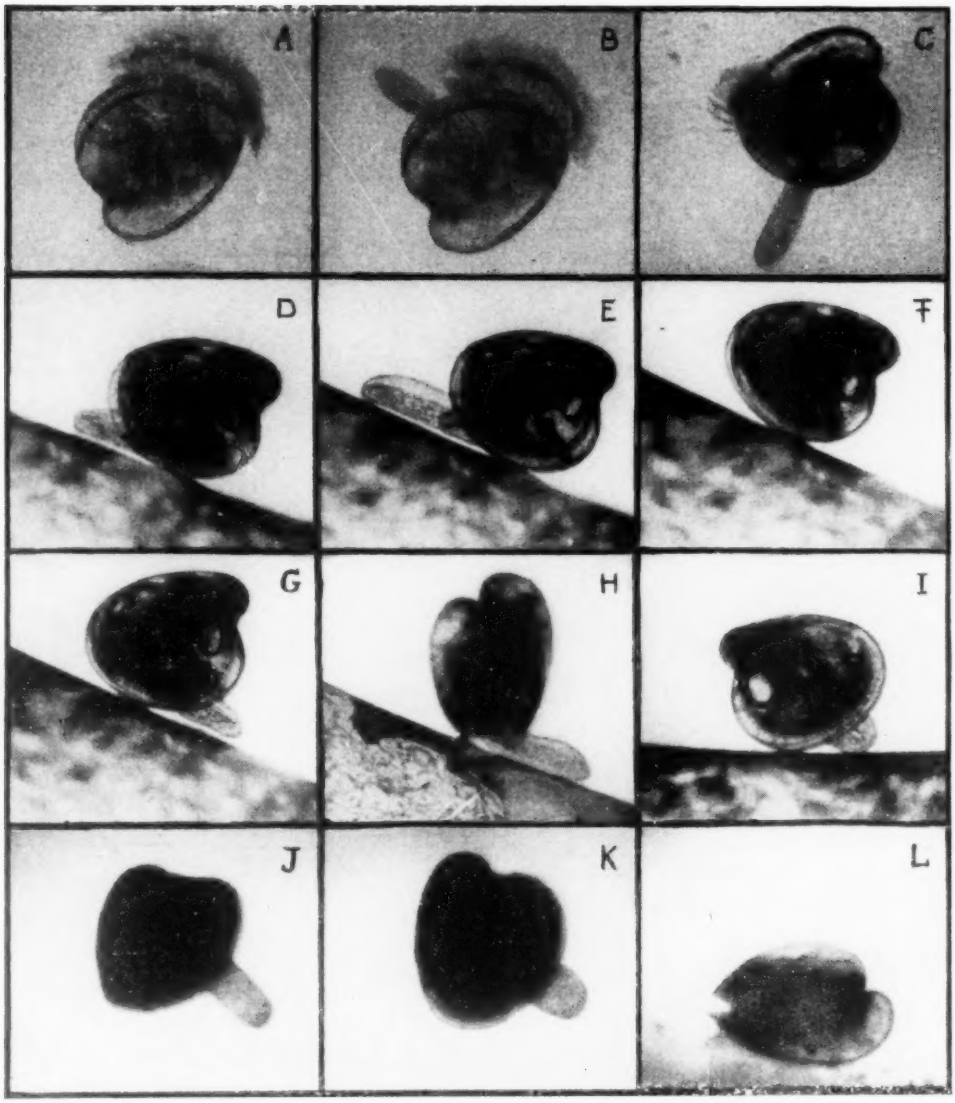

FIG. 3. Various stages of setting process taken from motion picture film. A, swimming larva; B, protrusion of foot; C, retraction of velum; D, larva crawling; E, foot fully extended (note position of byssal duct); F, G and H, larva reversing direction of crawling, shell held in position in F by byssus; I, preparing to release cement, (note reduced size of foot); J and K, larva setting with foot protruded from side of shell; L, attached larva, end view.

Water temperatures at surface and bottom were taken by means of a Negretti and Zambra deep sea reversing thermometer and additional records obtained from the long distance thermograph set up at the laboratory. In the experiments dealing with the effects of the neutral salts and heavy metals, the solutions were prepared in various known concentrations with copper free distilled water. Aliquot portions of each were then added to salt water containing the oyster larvae. Only C.P. chemicals were used and when nec-

essary particular care was taken to free them from traces of copper in view of its marked stimulating effect on setting.

In making quantitative copper determinations of sea-water or fresh water the method of Callan and Henderson (1929) was employed in which sodium-diethyl-dithiocarbamate is used as an indicator. This method is accurate to 0.1 mg. of copper per liter. For samples of lower concentration it was necessary to remove the copper from several liters by precipitation and filtration in order to have a sufficient quantity of the metal for analysis. The results by this method were checked occasionally with the potassium ferricyanide method and were found to vary less than 0.1 of a milligram per liter.

ANATOMY OF THE OYSTER LARVA

In northern waters the oyster larva attains complete development after a period of approximately two weeks. Studies of the morphology of the full grown larva were made to augment those on physiology and behavior as certain anatomical features are of particular importance in relation to setting and metamorphosis.

Stafford (1913) gives a fairly complete description of the anatomy of the larva, including the previous observations of Brooks, Horst, Jackson, and others. However, many important details of the structure and function of larval organs are lacking, and consequently further observations along this line were undertaken in order to better understand the nature of the setting process. Studies were made of fixed and sectioned material, whole mounts, and immobilized living forms. The latter were subjected to vital stains, narcotics and feeding of colored particles, such as carmine. The anatomy of the full grown oyster larva is presented in a semidiagrammatic way in Figure 4. This drawing shows the appearance of the larva as seen through the lower or left valve and was prepared from whole mount specimens that had been narcotized with Mg. SO_4 and chloroform, before fixation in modified Bouin's solution (B.3). Photomicrographs of active, living specimens are shown in Figures 3 and 5.

The oyster larva is covered with a thin, transparent, chitinous shell (Fig. 4, Sh.) composed of two valves, that are joined together by a hinge along the dorsal edge and also by the anterior and posterior adductor muscles. The valves are of unequal size, the left or lower one (which is cemented to the substratum during setting) being larger, more convex and having a more prominent umbo. The appearance of the two valves as seen in side view, is shown in Figure 3 H and L. The shell of a fully grown larva measures on the average 0.33 of a millimeter across its greatest diameter and has an average width of 0.22 millimeter. The two valves fit tightly together and the larva by retreating therein is able to protect itself from natural enemies such as protozoa or against adverse environmental conditions.

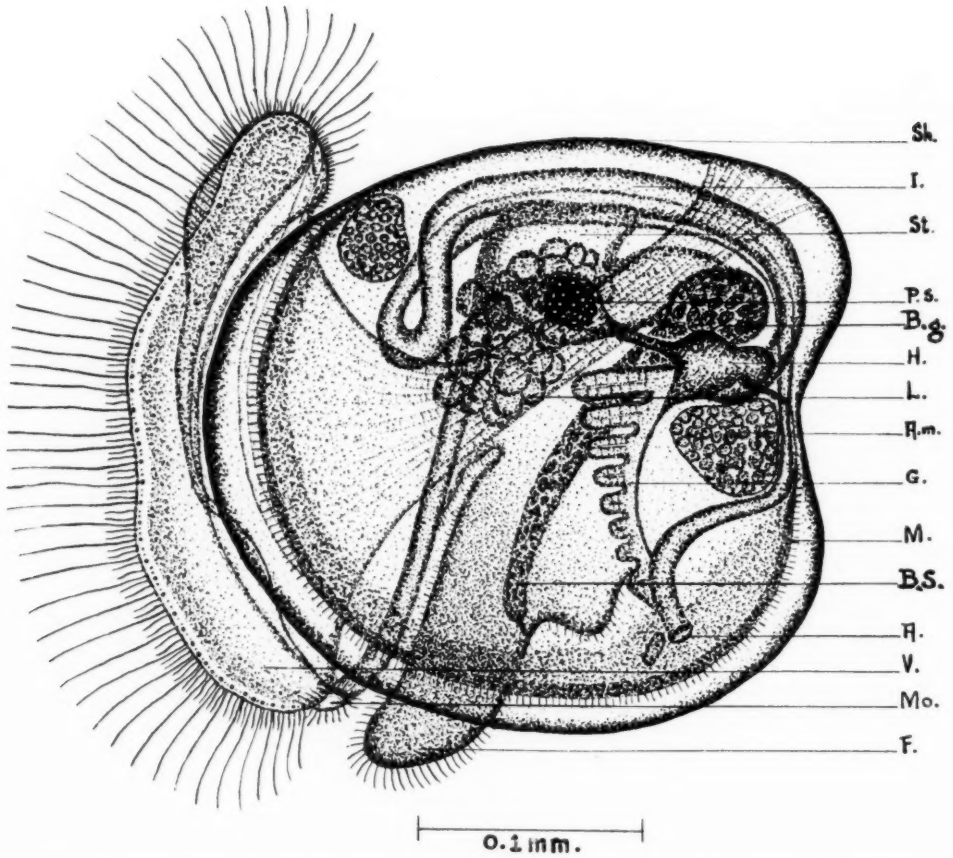

FIG. 4. Anatomy of oyster larva as seen from left side. Sh, shell; I, intestine; St, stomach; P.S., pigment spot; Bg, byssal gland; H, heart; L, liver; Am, adductor muscle (posterior); G, gills; M, mantle; Bs, byssal secretion; A, anus; V, velum; Mo, mouth; F, foot.

Surrounding the flesh of the larva is a thin transparent membrane or mantle, M, the outer edge of which is greatly thickened and covered with numerous large cilia. The mantle serves not only as an important organ for the deposition of the shell but is also a highly sensory and protective structure. The inner side of the mantle and nearly all exposed body surfaces of the larva are covered with extremely fine cilia which serve to keep them clean, and assist respiration by keeping the water in movement.

The two most important larval organs are the velum and the foot both of which disappear during metamorphosis. The velum is the primary organ of locomotion, feeding, and respiration, and is shown in a partly extended condition in Figure 4, V. The larva swims rapidly by means of the long powerful cilia which cover the outer edge of the velum and appear to be under the control of the nervous system. The beating of these cilia causes a strong current of water to pass over the velum which extracts from it both food and

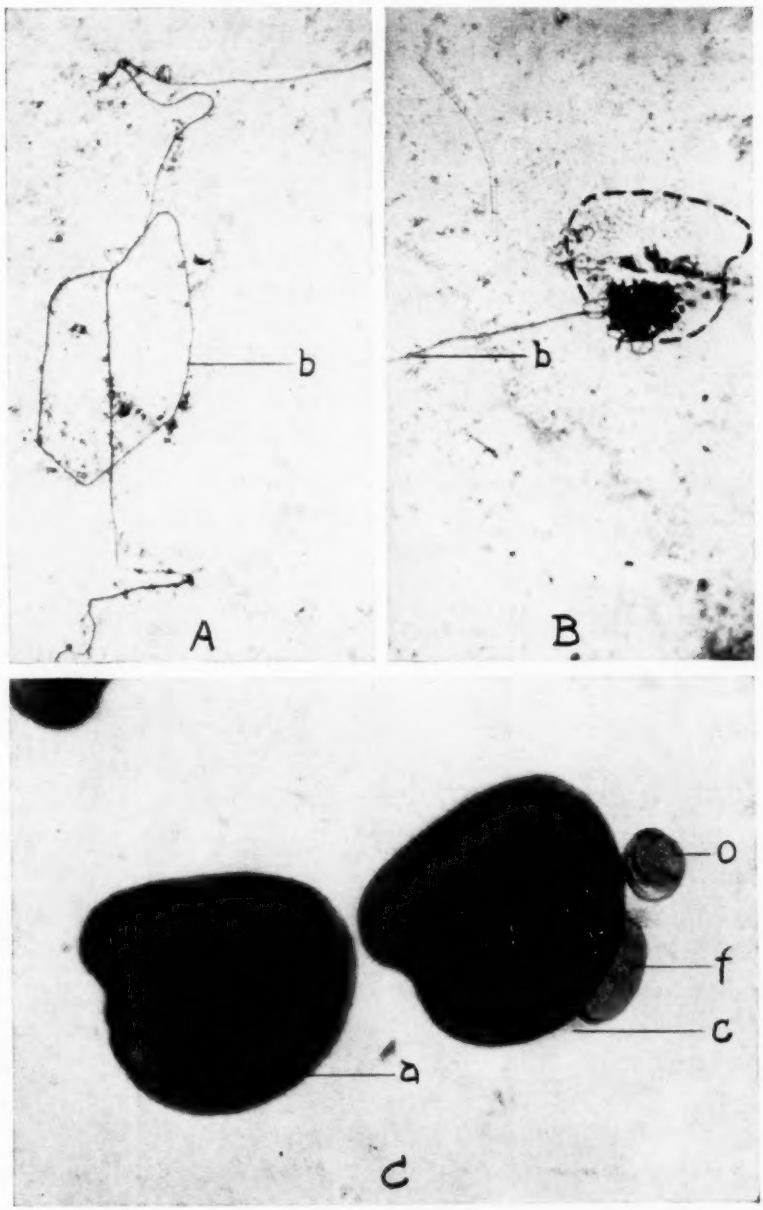

FIG. 5. Photomicrographs of setting. A and B, byssus trail at beginning and end of crawling period; C, larva in final act of setting as foot holds left valve of shell in cementing fluid; b, byssus; f, foot; c, byssal cement; o, one day old larva entangled in cement; a, attached larva.

oxygen. The velum is able to function in this respect whether the larva is lying on the bottom or swimming.

In locomotion the velum precedes and drags the heavy body suspended beneath and behind it, as shown in Figure 3, A and B. As seen from the front, the velum is elliptical in shape, thin at the margins and gradually be-

comes thicker and more rounded toward the center where it is attached by a broad stalk to the body. This stalk decreases rapidly in size as it passes into the shell and contains 14 cross striated muscle fibers, 7 of which pass on each side of the body and attach near the hinge. These fibers serve in retraction of the velum, and during setting were observed to break loose thus preventing further use of this organ.

In mature larvae the foot is a highly developed organ by which they are able to crawl under conditions where swimming is impossible. This organ Figure 4, F is brought into play chiefly during setting but may be used previously whenever the larva is surrounded by mud, sand, etc., and is in danger of being smothered. The larvae cemented to glass slides used the foot vigorously in an attempt to free themselves. The foot is extremely powerful and is used frequently toward the end of the free-swimming period when the animal is larger and heavier, and settles more often to the bottom.

During the process of attachment, the foot renders its greatest service in bringing the larva into contact with a submerged surface, crawling over it, and finally as its last function, holds the left valve into the cementing fluid as setting is completed. During setting, there is discharged through the bottom of the foot the secretion of the byssal gland which forms at first a microscopic, sticky thread or byssus that may be several inches in length. Later the remaining fluid in the gland is forced out in bulk and constitutes the cementing substance by which immobilization of the larva is accomplished.

Stafford (1913) concluded that the byssal gland is located in the foot and that its secretion is poured out through an opening in the posterior end or heel. The present studies fail to corroborate this view and show that the gland itself lies above the posterior adductor muscle as shown in Figure 4, *Bg*. The byssal gland is a large, rod shaped organ lying across the posterior end of the larva. Two, long, tapering ducts lead down from the gland into the foot. These ducts are located on each side of the body and empty the byssal fluid just anterior to the heel, through two, small converging tubes. These pass into the median grove along the bottom of the foot where the byssus is formed. In preserved larvae, the byssal secretion (Figure 4 *Bs*) appears as a large mass of amber colored globules of varying size which have a marked affinity for the hematoxylin stain.

The organs of feeding and digestion are highly developed and consist of the velum, mouth, esophagus, stomach, intestine, and digestive diverticula. The food is extracted from the water by the small velar cilia and carried ventrally into the mouth (Figure 4 Mo). The digestive tract is lined with cilia which keep the food and other particles in continuous movement through it. When larvae are placed in a solution containing carmine in suspension they quickly gather it into the mouth and pass it through the entire digestive tract in a period varying from 4½ to 6 minutes.

From the mouth the food passes through a long esophagus into the stomach (Fig. 4 *St*) where it can be seen in active rotation with the crystalline style, as digestion takes place. From here it is carried posteriorly into the intestine (Fig. 4 *I*) which is a long slender tube that passes forward to the anterior adductor muscle and there turns about and runs backward and behind the posterior adductor muscle near which it ends in a short cloaca and anus. The cloaca expands and contracts regularly as it becomes filled with digested material and forcibly ejects it from the body.

Surrounding the lateral and ventral portions of the stomach and communicating with it are numerous, brown sacs or the so called "liver" of the larva (Fig. 4 *L*). These blind ciliated tubules are according to Yonge (1926) digestive diverticula and function as organs of assimilation and intracellular digestion. He states that they are the only portion of the digestive tract where soluble food materials are absorbed. Our experiments indicate that these tubules are organs in which there is storage and activity of digestive enzymes. In vital staining experiments with neutral red at a concentration of 1 to 10 millions these were the only structures in the body which stored the dye in noticeable amount. This is an indication according to Koehring (1930), that these digestive cavities have a high enzyme content.

Pigment spots are found on each side of the body as very conspicuous larval organs and consist of a dense mass of cells containing a dark blue-green pigment. These spots (Fig. 4 *Ps*) are formed gradually during larval development and disappear quite rapidly after setting, when they break apart into cells which migrate into the blood stream. The behavior of these pigmented cells and their function and identity in the development of the oyster is discussed later in the studies on the relation of copper to metamorphosis. Frequently in the literature the pigment spots have been incorrectly called eyes or eye specks and considered as light sensitive organs. No evidence has been found by the author in various experiments over a period of 8 years, which indicates that oyster larvae are sensitive to variations in light intensity or color.

The circulatory system of the larva is difficult to discern in respect to both structure and function. In larvae which were artificially cemented to glass slides the slow movement of a clear fluid in the blood vessels could be seen, though there were no noticeable pulsations of the heart. The main blood vessels leading from the heart were those which passed to the rudimentary gills and to the pigment spots on each side of the body. Heart beat and more rapid circulation of blood first became noticeable immediately after metamorphosis. At a temperature of 25° C., the heart in a small spat beats at a rate of 110 to 114 pulsations per minute. At the same time a strong flow of blood, containing leucocytes can be observed in the blood vessels.

The gills of the larva consist of two plates that are located on each side of

the body just outside of the upper portion of the foot and in front of the posterior adductor muscle. Each plate is made up of a series of eight, short papilla like processes as shown in Figure 4 G which diminish in size from the dorsal portion downward and are best developed on the left side of the organism.

The most important muscles are the anterior and posterior adductors which extend transversely between the valves and serve in drawing them tightly together. The posterior adductor (Fig. 4 *Am*) is the larger of the two and develops rapidly into the large shell muscle of the adult. The anterior adductor which is nearest the velum undergoes almost complete degeneration after metamorphosis. There are also numerous, slender, retractile muscle fibers lying in the mantle, velum and foot, which converge and are attached inside of the two umbos. These serve in the retraction of these organs and function especially during the vigorous crawling movements of the setting larva.

THE SETTING PROCESS

The act of setting can only be initiated and completed when the organism has reached complete larval development. This condition can be determined from the size of the shell, the volume and density of color of the two pigment spots, and the occasional protrusion of a well developed foot as described previously.

The setting process which is shown diagrammatically in Figure 6 may be divided into three, fairly definite, continuous phases; namely, searching, crawling, and fixation. During the first phase the larva protrudes the foot to nearly its full length and swims rapidly through the water in search of some solid object. Generally, the larva swims in the usual spiral manner waving the foot about and releasing from it a fine sticky thread or byssus which serves to anchor it to any shell or other surface with which it may come in contact. However, in many cases the larva, immediately after stimulation swims in a straight line at unusually rapid speed, holding the foot foremost so that the byssus secretion is the first point to come in contact with the submerged structures which it encounters.

Frequently, the larvae failed to go through the searching phase and merely dropped to the bottom of the dish, withdrew the velum, protruded the foot, and began crawling. This type of behavior occurred largely in experiments where the copper content of the solution was greater than that needed to initiate setting so that the reactions of the larvae were somewhat forced. However, under natural conditions the amount of copper present is exceedingly minute so that normally the larva goes through a short searching phase. This phase may be defined as the interval from the first protrusion of the foot to the retraction of the velum when crawling begins. Its duration may vary

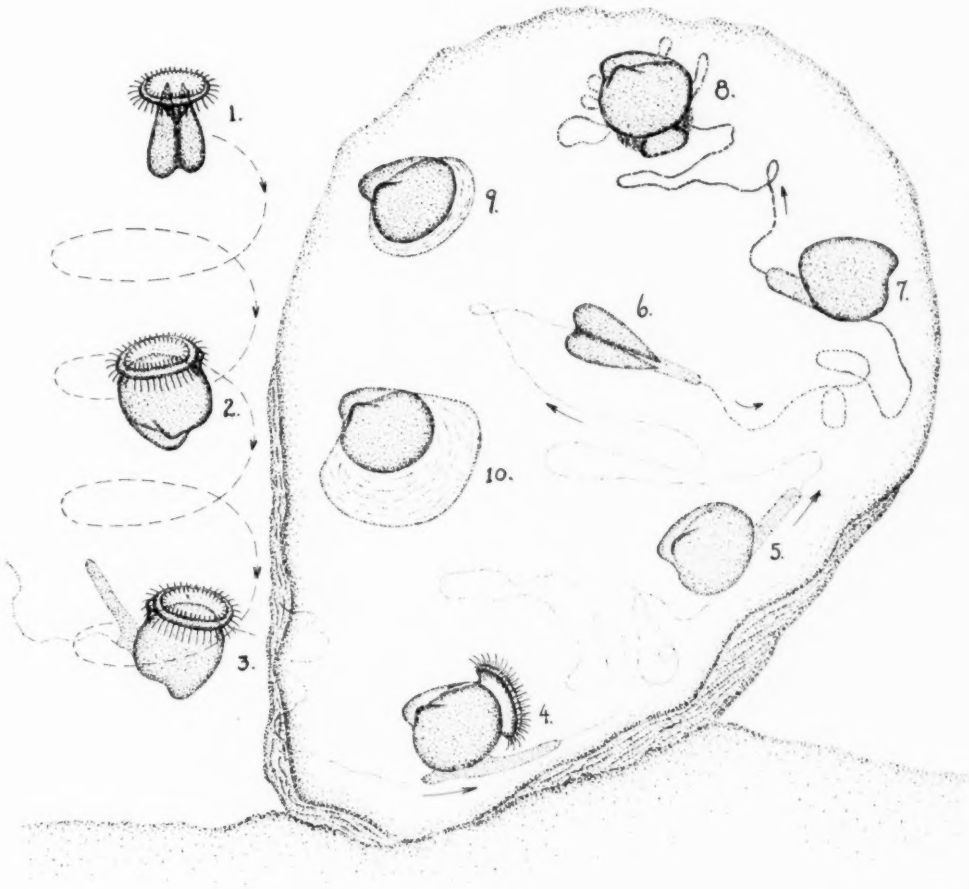

FIG. 6. The setting process (diagrammatic, proportionate size of larvae greatly increased). 1 and 2, swimming larvae, side and end views; 3 and 4, searching phase; 5 and 7, crawling phase; 8, fixation to substratum; 9 and 10, spat one and two days old.

from less than a minute to an hour or more according to salinity, temperature, maturity of the larva, and the nearness of a suitable surface for attachment. The searching phase lasted for 2 minutes in solutions having a salinity of 16 parts per million and temperature of 23° C.

After the larva has established contact with some firm surface by means of the byssus and foot, the crawling phase begins as shown in Figure 6 numbers 4 to 7. The velum is soon drawn into the shell and the larva proceeds to crawl vigorously and rapidly over the surface by means of its long, powerful foot. In crawling, the foot is extended to its full length and the anterior end held in contact with the surface while the remaining portion contracts suddenly and pulls the shell of the larva forward. This process is continually repeated while at the same time the byssus is being drawn out from the bottom of the foot and adheres closely to the underlying surface. At first the larva crawls quite rapidly at a rate of approximately 2.5 cm. or 1 inch per

minute when it experiences little difficulty in pulling the shell after it or in drawing out the byssal substance. From this time on the crawling becomes gradually slower and slower and is most sluggish just before setting when the larva moves back and forth very slowly over the small area to which it becomes attached.

At the beginning of the crawling phase the larva moves for a considerable distance in almost a straight line, until on reaching the edge of the object or some obstruction, it turns about and proceeds in the opposite direction. Later the larva reverses its direction in crawling more frequently and as the time of setting approaches, moves but a few millimeters back and forth in one spot. In order to reverse its direction in crawling, the larva draws the foot into the shell and protrudes it from the posterior end after which the shell and body are spun around to the left and crawling resumed as before.

During the crawling phase the foot gradually changes from a long and slender shape to one that is short and broad as shown by the photomicrographs in Figure 3 and 5. The length of time that the larva crawls may vary from 12 minutes to several hours according to the salinity of the water as discussed in greater detail later. The end of the crawling period can easily be recognized by the slow, forced movements of the larva, the shape of the foot, and the frequent backward and forward crawling of the larva over an area scarcely a millimeter in diameter.

During the entire crawling process, the byssus is being released and tenaciously adheres to the surface over which the larva is moving so as to prevent its dropping to the bottom or being washed away. A photomicrograph of the byssus trail is shown in Figure 5 together with the final byssal secretion that cements the larva to the substratum.

At first the byssus is fairly heavy and cylindrical in shape with a diameter of 0.004 millimeter but gradually becomes thinner and flattened to a width of 0.01 millimeter as setting is about to occur. Judging from the behavior of the larva and its speed and effort in crawling, it is apparent that the byssal material is released with increasing difficulty as times goes on and that frequent contractions of the shell against the foot are necessary to force out this secretion.

The total length of the byssus or the maximum distance covered by the crawling larva was found to vary from a few inches to 15 or 20 inches according to the duration of the crawling phase and the nature of the substratum. The temperature and salinity of the water have a marked influence on the velocity of the setting reaction and particularly on the length of time that the larva is crawling. The nature of this effect in relation to the secretion of the byssus is discussed later. The crawling phase is by far the longest period of the setting process and is terminated very suddenly as the larva quickly cements itself to the substratum.

Finally, as the movements become slower the larva comes to a standstill holding the shell at right angles to the surface preparatory to attachment. Next, the foot is quickly protruded to its full length from the right side of the shell at about the center and flattened down against the surface (Fig. 3, *I* to *K*). Then as the shell is pulled a short distance sideways by short jerky movements, there follows one or two vigorous contractions of the valves against the base of the foot which force out the cementing fluid or last of the byssal substance. The shell is then quickly and forcibly pressed down against the surface so that the left valve of the larva is tightly held in contact with the cement which hardens almost immediately. The foot holds the larva firmly in place for a minute or two and then is slowly withdrawn into the shell completing the process of setting (Fig. 5 *C*). Tests made one minute from the time of withdrawal of the foot showed that the cement had already hardened to such an extent that the larva could not be detached by vigorous washing.

These observations on the setting process, made on several hundred individuals under various conditions, differ in many respects from those reported by Nelson (1924). Nelson's description is based on a single experiment in which six larvae were setting simultaneously. He describes the larvae as crawling in circles and assumes a tendency on their part to keep separated from one another thus producing a fairly even distribution of spat and greater room for growth. The present studies show that the larvae do not describe circles during the crawling phase but move back and forth in a zigzag manner forming an irregular star shaped path. The larvae exhibit no decided tendency to avoid each other and in chance encounters are likely to set in contact with or upon one another. In fact the larvae on striking an obstruction or depression show a tendency to remain and set there where circumstances are often less favorable for growth. Only one or two contractions of the foot would move the larvae 0.5 mm. or more away from another individual and consequently it is not surprising that Nelson finds but a small percentage (11.4 per cent) within this distance of one another. However, with a heavy set such as is shown in Figure 1 over 98 per cent of the larval shells are attached within 0.5 mm. of each other and many are in contact or but 0.1 to 0.3 of a millimeter apart. The surface area is obviously limited in proportion to the number of spat though other portions of this same shell are comparatively barren. It is apparent that the larvae do not exercise intelligent selection of a setting location and that there is little evidence for Nelson's conclusion, that, the frequency of attachment per unit area of available cultch does not exceed a certain maximum, though there are high concentrations of larvae in the water.

Nelson states that by the use of the foot the larva is able to exercise some selection in seeking a place for attachment and that it avoids surfaces that are microscopically rough. However, it was observed by the author that the

larva covers an appreciable area during the crawling phase if the surface is smooth but its final location is largely a matter of chance rather than choice. On rough surfaces the larvae attach more readily and crawl but a comparatively short distance. The cement coating on the partition collectors shown in Figure 2 is very rough which makes it ideal for the attachment of the larvae. Smooth glass slides and china were placed near and inside of these collectors and gathered less than 0.1 as many spat, while ground glass worked nearly as well as the cement surface.

The behavior of the larva during the final phase of setting is described by Nelson (1924) as follows: "With the foot extended to about one half its full length, its distal end flattened in contact with the glass, the larva swung the body until the foot occupied the median position with the left valve against the glass and inclined to it at about 30 degrees." The author's observation differed from these in that the foot, which had now become short and broad, was protruded to its full length from the right side of the shell near the center thus making the twisting of the body and shell unnecessary. At first the shell was held at right angles to the surface as the cement was being secreted and then was slowly pressed into this fluid until the left valve was inclined at an angle of approximately 45 degrees.

According to Nelson (1924) the mantle is extended by the larva for a short period (2 minutes) as an aid in the distribution of the cementing fluid. No evidence in support of this fact was found by the author. The present studies show that the larva merely pressed its shell slowly into the cement while the ventral edge of the left valve was in contact with the substratum, and this movement was sufficient to force the cement back under the shell for some distance.

On numerous occasions the larvae were observed crawling about on the under side of the surface film in much the same way as they would over glass. The byssus was laid down in the usual manner and finally the larvae would cement themselves to this film and thus remain suspended for a short time at the surface of the water. In surface plankton collections a small number of newly formed, unattached spat were occasionally found, apparently as the result of the larvae attaching to the surface film in this manner.

ORIENTATION AND VERTICAL DISTRIBUTION OF SPAT

When oyster larvae set upon a vertical surface, they invariably place the shell with the dorsal edge or hinge uppermost as shown in Figure 6 numbers 8 to 10. Then the shell grows gradually in a spiral clockwise manner around the posterior end of the spat until in a few weeks the long axis of the shell and position of the internal organs is nearly the reverse of what it was at the time of setting. Spat on a horizontal surface show in many cases this same

spiral movement in shell growth though it is much less pronounced than in the former instance.

Two experiments were carried out to determine how the oyster larvae would distribute themselves when induced to set in glass aquaria. In the first experiment, over 1,500 larvae were placed in a 2-liter rectangular glass jar containing a slowly circulating mixture of sea-water and river water in which there was sufficient copper to stimulate setting. The second experiment was made under similar conditions using a tall, cylindrical glass vessel of 2 liters capacity in which over 10,000 larvae attached themselves over a period of 24 hours. The distribution and concentration of the spat on the sides of these aquaria is shown graphically in Figure 7. In these solutions the majority of the larvae settled gradually to the bottom of the vessels and there began crawling. These movements soon brought them in contact with the sides of the jar where they proceeded to crawl upward for distances varying from an eighth of an inch to several inches.

On the bottoms of the jars the intensity of setting per unit area was higher than on the sides because of the lesser surface area presented by the former. In both experiments a considerably larger number of larvae were found attached to the sides of the vessels. In the first experiment only 20.3 per cent of the total number of larvae attached to the horizontal bottom surface while the majority, 79.7 per cent, set upon the vertical side area. In the second experiment 15.3 per cent of the larvae set on the bottom of the cylindrical jar and 84.7 percent on the side. The percentage distribution of spat in the latter experiment is given in the following table.

TABLE 1. Distribution of spat in glass jar (2nd experiment).

Zones	Total spat per zone	Percentage of total number of spat
Bottom	1,532	15.32
0 to 1 inch	2,535	25.35
1 to 2 inches	1,914	19.14
2 to 3 inches	1,300	13.00
3 to 4 inches	924	9.24
4 to 5 inches	647	6.47
5 to 6 inches	561	5.61
6 to 7 inches	343	3.43
7 to 8 inches	178	1.78
8 to 9 inches	66	0.66

Table 1 shows clearly the tendency of the larvae to crawl upward on inclined or vertical surfaces, which may be due to a negatively geotropic response of the organism during the crawling period. It is also possible that the weight of the shell is responsible for this climbing movement as the larvae appeared to be better able to handle the shell and drag it behind them when moving upward than when crawling horizontally. Over 50 per cent

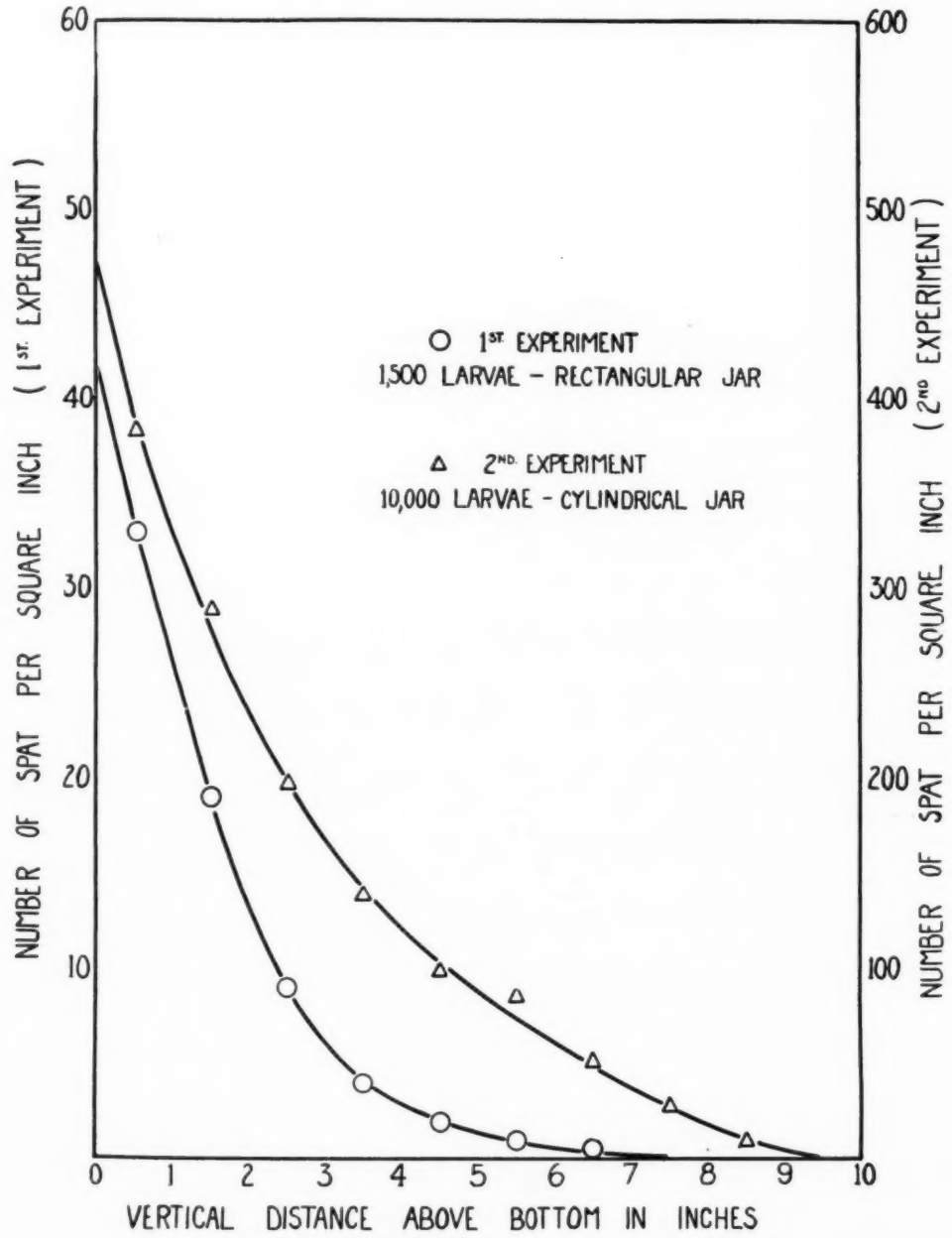

FIG. 7. Vertical distribution of spat in glass aquaria.

of the larvae set in the zone extending from 1/8 of an inch to 3 inches above the bottom. Under natural conditions this upward movement would in most cases be decidedly favorable to their survival as it would result in sufficient elevation to prevent their being smothered by sedimentation or a slight settling of the cultch into the bottom.

RELATION TO ENVIRONMENTAL CONDITIONS

Oysters are distributed in inshore areas along the coast where mixing of sea-water and river water is constantly taking place and consequently their environment is exceedingly complex from a physical and chemical standpoint. In some localities where the beds lie between tide marks or in shallow waters, there are extreme fluctuations in temperature, salinity, hydrogen-ion concentration, current velocity, etc. as a result of variations in the amount of river discharge and the horizontal and vertical movement of the water by the tide. The regions or zones in which these fluctuations are the most pronounced are primarily the prolific seed producing areas. Here setting reaches its greatest intensity and we find the maximum number of oysters attached per unit of surface as shown by Prytherch (1929) and Galtsoff, Prytherch, and MacMillin (1930). Though the vertical and horizontal distribution of oysters and the location of heavy setting zones showed marked differences in the various localities, nevertheless, these zones can be correlated in each case with certain similar changes in the physical condition of the water. Studies by the author in Milford Harbor, Conn. in 1925 and 1926 show that the oyster larvae are most abundant during low water periods and that setting occurs in greatest intensity at such times. Thus by an analysis of the tidal conditions in the various oyster regions it was possible to correlate the distribution of oysters with the velocity of the currents and abundance of the larvae, the heaviest setting in each case occurring at the levels where the larvae were plentiful as the current reaches its minimum velocity.

To analyze further the connection between current velocity and setting a special study was made in 1927 in Folly River and Charleston Harbor, S. C. of the relation between the vertical distribution of oysters and changes in the physical condition of the water. These studies indicated that a parallel existed between current velocity and changes in salinity and that heaviest setting occurred at the stage of tide when the salinity was lowest. This fact was further emphasized by previous and subsequent studies in Milford Harbor, Conn. by the author and in Onset Harbor, Mass. by Galtsoff and MacMillin. In dealing, however, with the relation between low salinity and heavy setting, it is apparent that at such times there is also the maximum effect of river discharge on the physical and chemical condition of the water over the oyster beds. Several facts indicate that the lowering of salinity by river discharge had a pronounced effect upon the setting of oyster larvae; namely, first, that the natural beds and heavy setting or seed producing areas were located adjacent to or in the mouths of rivers; second, that setting occurred during the period of neap tides when the inflow of ocean water was at a minimum and the salinity reduced to a greater extent by river water; third, that in Milford Harbor attachment of larvae in greatest numbers occurred in the surface layer during the low water stage of the tide; and fourth, that during

seasons of excessive river discharge there was a marked seaward extension of the areas on which setting of oysters takes place.

Milford Harbor is a very favorable oyster setting basin in which all variations in conditions from fresh water to that of a salinity of over 28 parts per thousand can be obtained within a distance of 3 miles. A considerable variation in water conditions can likewise be obtained frequently during a complete tidal cycle at the point where the laboratory is located. At the time that setting was in progress in 1928, 1929, and 1930 several series of observations were made from high water to low water and back again which exhibit variations of the type shown in Figure 8. These samples were collected hourly always at the same level or point, the location of which was 1 foot below mean low-water mark and coincided with the lower portion of the zone in which larvae, spat, and adult oysters are most abundant. Consequently the fluctuations in water conditions shown in Figure 8 are representative of the changes that occur at this tidal level.

At the time of low water the concentration of salts is approximately one half of that of the water in Long Island Sound which flows into this harbor. This means that there had been a mixture of approximately equal volumes of river and sound waters so as to bring about a 50 per cent reduction in salinity at the time that setting occurred. The factors of salinity and river discharge appeared to have the greatest influence on setting and consequently laboratory studies were carried out to test experimentally the effect of these conditions on the larvae.

It was found that setting was not induced by changes in salinity or by any specific salt concentration though, as is shown later, this factor did affect the duration of the setting process. These experiments, however, supplied two excellent clues as to the principal factor governing setting. When the salinity was reduced with river water instead of distilled water, the larvae gave a positive setting reaction which indicated that some change or substance had been introduced by the river water that served to stimulate and induce setting. This observation was preceded by a most fortunate accident as a result of which the salinity samples were contaminated with minute traces of copper as they were run through a centrifuge for the removal of plankton, sediment, etc. The solutions before centrifuging had no effect on the larvae, while afterward they produced immediately the setting reaction because of the trace of copper that had been dissolved by the solution as it passed over a small uncoated portion of the centrifuge.

Thus on August 5, 1928, it was determined for the first time that copper would induce setting of the oyster larvae. By the careful use of this element in minute amounts it has been possible to observe the behavior of thousands of larvae during this process as described previously. In making detailed observations on setting the mature larvae were placed in a Syracuse glass

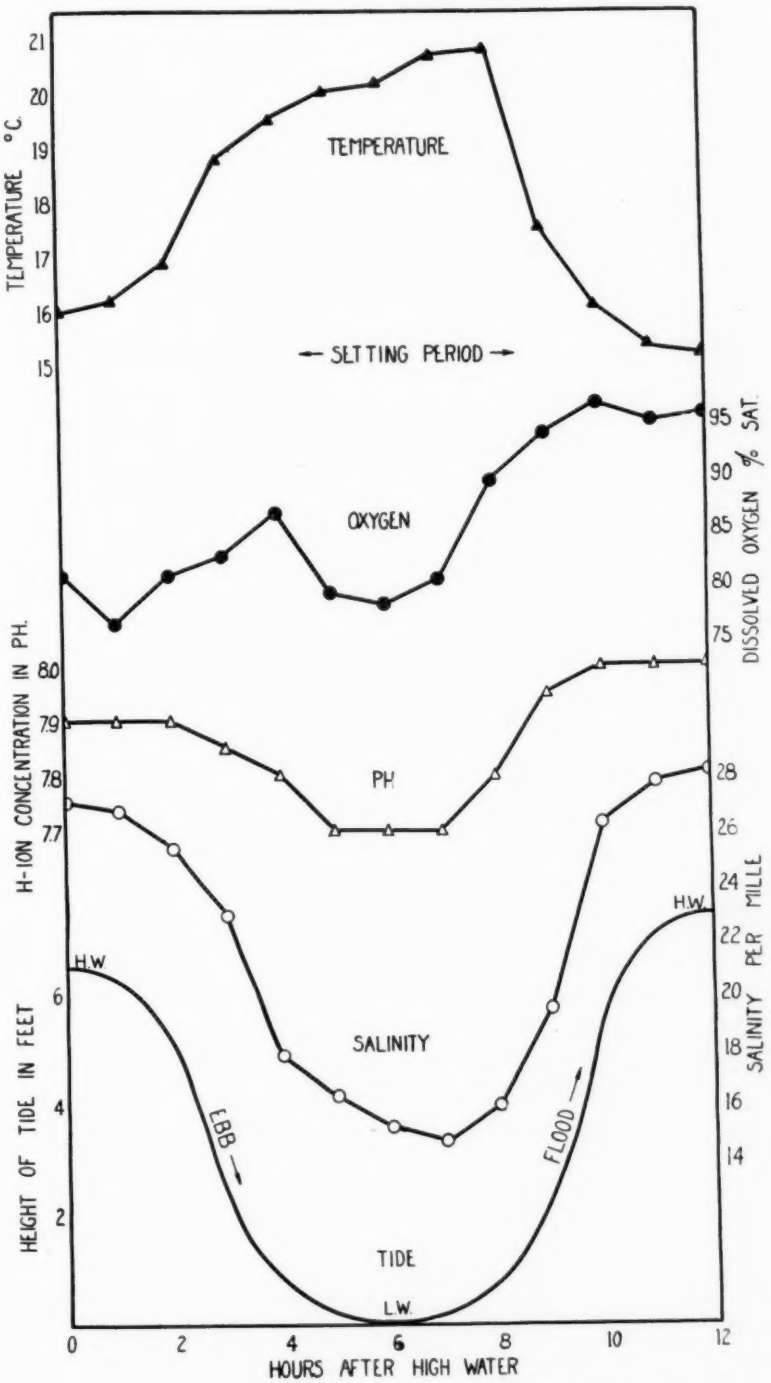

FIG. 8. Hourly fluctuations in salinity, pH, oxygen and temperature during complete tidal cycle, July 12, 1928, Milford Harbor.

containing 10 c.c. of sea-water (salinity 15-20 per mille) and a small piece of metallic copper 0.5 cm. square, dipped in the solution for an interval of 15 to 30 seconds. Under these circumstances it was found that the amount of copper dissolved from the metal was exceedingly small so that its effect on the larvae was of a stimulative rather than toxic nature. The larvae reacted almost immediately to the presence of copper and began the series of performances which ultimately led to their immobilization.

In Connecticut the oyster beds have suffered serious damage from copper laden trade wastes, and it seemed highly improbable that this metal was the important element operating under natural conditions in the setting of the oyster. Consequently for two years every possible attempt was made to find some other substance or physico-chemical factor that would induce setting.

Before proceeding with the analysis of the various physical and chemical factors in relation to setting, it will be best to review first the studies of the effect of salinity on the duration of the setting process.

TIME FOR COMPLETION IN DIFFERENT SALINITIES

Under natural conditions the setting of oyster larvae in different localities occurs in waters having a wide range of salinity while other factors such as temperature, pH, etc. show little variation. For example, in the Chesapeake Bay region the best setting areas are located not only in the mouths of the contributing streams where the salinity ranges from 5 to 15 parts per thousand but also along the ocean shore where the water has a much higher salinity of 27 to 30 parts per thousand. In other coastal regions, the salinity in many of the heavier setting areas is approximately as follows:

TABLE 2. Salinity of water in oyster setting areas.

Locality	Salinity Per Mille	Reference
Onset Harbor, Mass.	24–30	Galtsoff, Prytherch, & McMillin (1930)
Wareham River, Mass.	26–29	Galtsoff, Prytherch, & McMillin (1930)
Milford Harbor, Conn.	25–28	Prytherch (1929)
Stratford Point, Conn.	26–29	Prytherch (1929)
Great South Bay, L. I.	14–19	Prytherch (unpublished field observations, 1925)
Delaware Bay, N. J.	10–22	Nelson (Delaware Diversion Report, Unpublished)
Upper Chesapeake Bay, Md.	7–15	Truitt (1920-25)
James River, Va.	11–20	Prytherch (unpublished field observations, 1930)
Newport River, N. C.	12–26	Prytherch (unpublished field observations, 1931)
Beaufort, S. C.	25–30	Galtsoff and Prytherch (1927)
Doboy Island, Ga.	10–28	Galtsoff and Luce (1930)
Galveston, Texas	8–24	Hopkins (1931)

In some localities the salinity may fluctuate considerably according to the hydrographical conditions, range of tide, and amount of river discharge while in others it remains relatively constant. It was desirable, therefore, to

know how waters of different salinity affected the larva in respect to the length of time for attachment and what was the optimum salt concentration for this process.

Large numbers of larvae were placed in natural sea-water of different salt concentration, ranging from 3.77 to 40.25 per mille, for a period of 24 to 96 hours previous to the time of observation. The solutions were prepared by evaporation and by addition of distilled water to a standard solution having a salinity of 28.31 per mille. During these experiments the pH of the water varied from 7.9 to 8.1 and the temperature from 22.9° C. to 23.8° C., but fluctuations of this magnitude have no noticeable effect on the duration of the setting process.

The larvae were stimulated by the addition of very small amounts of metallic copper as described previously, after which observations were made on the length of time required to complete attachment. The duration of the setting process varies considerably in waters of different salinity as shown in Figure 9. The curve is based on direct observations of the setting of over 450 larvae. Many of the records coincide with the points shown in Figure 9, especially those obtained at intermediate salinities ranging from 15 to 25 per mille. The most rapid setting was observed at salinities of 16 to 18.6 per mille and was completed in from 12 to 19 minutes. In solutions that were above or below this salt concentration, the time for setting increased and reached a maximum of 140 to 144 minutes in salinities of 5.6 and 32.2 per mille respectively. Beyond these extreme salinities, no setting occurred though many of the larvae crawled for periods of over four hours but failed in the end to attach themselves.

It is to the advantage of the larva that setting be accomplished as quickly as possible before there is a sudden change in environmental conditions that may interfere with the successful completion of this process. Consequently, these experiments show that the optimum salinity for setting is found in salt concentrations ranging approximately from 15 to 25 per mille. This is shown more clearly in Figure 10 in which the plotted data in Figure 9 has been averaged so as to show the velocity or rate of setting in relation to salinity. Setting is accomplished, also, with considerable regularity in salinities ranging from 9 to 29 per mille while beyond these limits only a very small percentage of the larvae are able to complete this process.

In the different salt concentrations there was no evident acceleration or retardation of the swimming and crawling activities of the larvae to which the variation in time for setting could be attributed. The salinity seemed to affect primarily the functioning of the byssal gland, the secretion of the byssus and particularly the final discharge of the cementing fluid by this organ. In solutions of relatively high or low salinity an extremely fine and delicate byssus was formed more or less discontinuously and the larva seemed

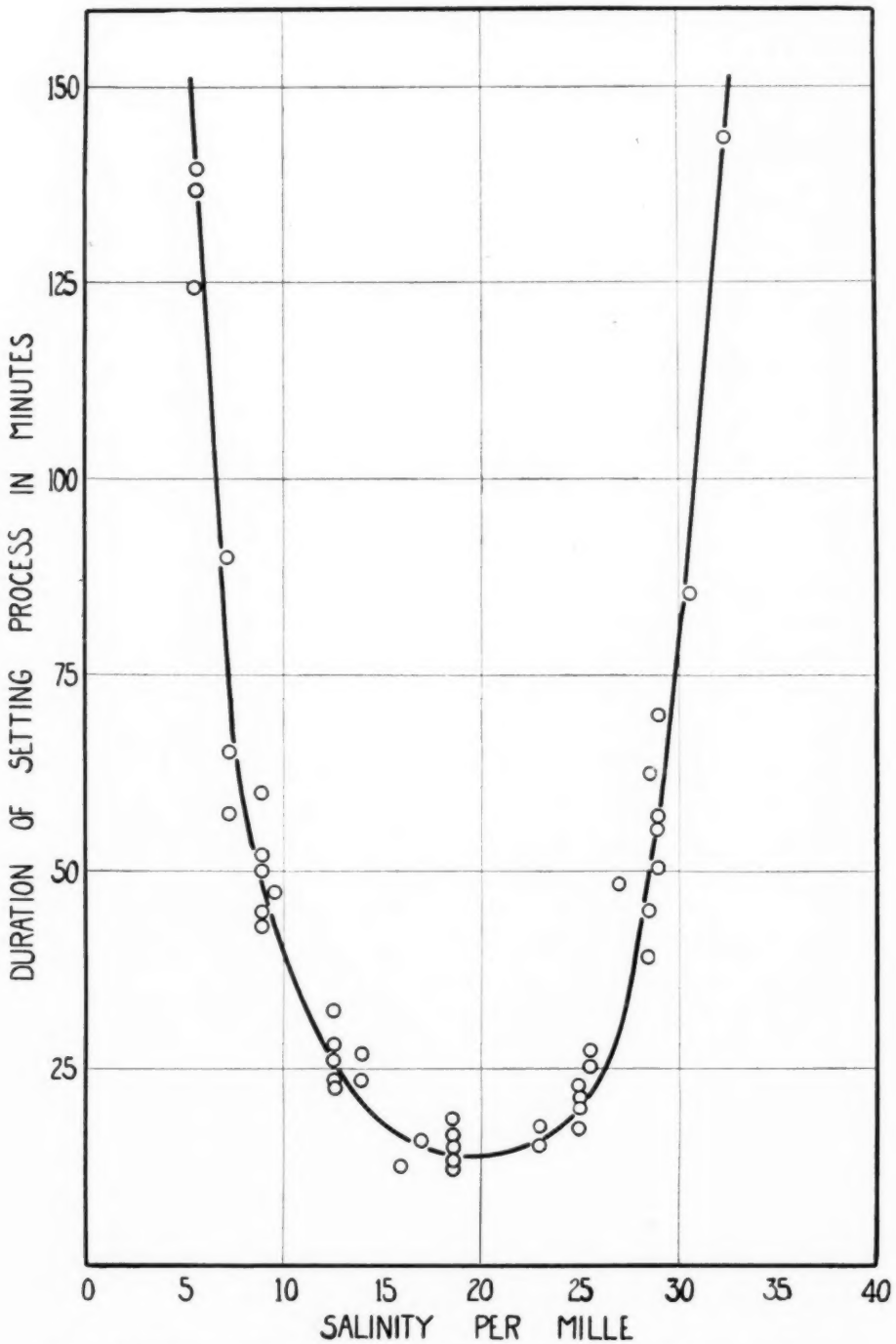

FIG. 9. The duration of setting process in waters of different salinity.

to experience considerable difficulty in ejecting the remaining fluid for attachment. On the other hand, in the intermediate salinities of 9 to 29 per mille, a very strong, sticky and elastic byssus of increasing size was laid down

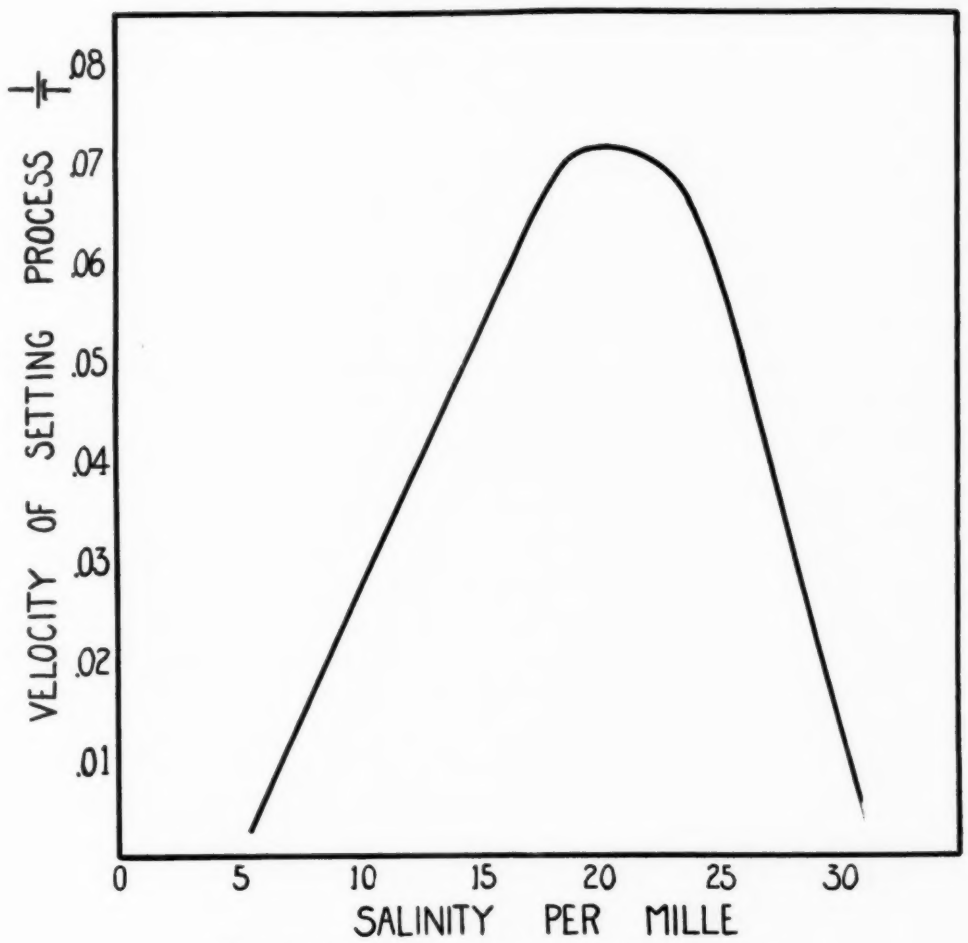

FIG. 10. Velocity of setting in relation to salinity.

during crawling as shown in Figure 5 and was followed by a sudden and easy discharge of the cementing fluid.

The chemical composition of the byssus material has been studied in the sea mussel *(Mytilus edulis)*, and other lamellibranchs, and as summarized by Field (1922) consists of an albuminoid material which gives a typical protein reaction. This substance is an organic colloid that is very resistant chemically to water, salt solutions, acids, and bases. The observed changes in physical properties of such a colloid in solutions of the neutral salts present a reasonable interpretation as to how the salinity may affect the secretion of byssal material. For example, it has been found that as the concentration of dilute salt solutions is gradually increased the viscosity of this type of colloid slowly drops to a minimum and then becomes greater again, so as to present a curve of the type shown in Figure 9. The byssal colloid of the oyster larva appeared least viscous and flowed most readily at salinities around 20 per mille while above or below this salt concentration there was a noticeable

increase in its viscosity. Therefore, we may interpret the effect of salinity on the velocity of the setting process as due primarily to a physical change in the byssal fluid, as a result of which the time for its complete discharge is altered.

The heavy setting of oysters under natural conditions was in conformity with these results as the salinity in these areas, as shown in Table 2, fell within the observed limits for successful termination of this process. The salinity range in Great South Bay, Delaware Bay, upper Chesapeake Bay, and James River comes the closest to the optimum concentration for setting and these are some of the greatest seed oyster producing regions in the world. In Great South Bay, the heaviest known setting has been observed with a concentration of spat on the shells varying from 500 to 1,000 per square inch.

The observations of Hopkins (1931) deserve consideration in this connection as they apparently show a correlation between increase in salinity and intensity of setting. His results indicate that setting periods follow rises in salinity above approximately 20 parts per thousand, and that completion of larval development is not attained unless the water reaches this higher salt concentration. This conclusion is not fully supported by his data which show that light setting did occur at salinities of approximately 8 to 16 per mille in Offatts Bayou (p. 68, fig. 10), and at salinities of 6 to 12 in June and 15 to 21 in July at Deer Islands (p. 69, fig. 11). An interval of heavy setting is also shown at a salinity of approximately 12 per mille for East Bay (p. 70, fig. 12).

It appears to the author that intensive setting in Galveston Bay and vicinity during periods of higher salinity is merely a coincidence resulting from the completion of larval development at such times. The oyster larvae collected here either differ markedly in size from those elsewhere or have been confused with other bivalve larvae. The measurements of the largest larvae by Hopkins (pp. 61 and 62) show a length of only 200 microns while in other regions oyster larvae of setting size have a length of 300 to 350 microns. Hopkins shows a heavy setting period in May at Offatts Bay (p. 68), while the largest larvae collected there at that time measured only 150 microns and could hardly have been developed to setting size. Similarly he shows periods of heavy setting at Deer Islands (pp. 62 and 69) when the majority of the larvae were less than 170 and 185 microns long. Since these plankton collections were made only at the surface it is likely that a complete picture of larval occurrence, size and growth was not obtained. In several localities in New England the bureau's investigators have been able to collect only a very small percentage of larvae where heavy setting occurs and such a condition may also be responsible for the results obtained in Galveston Bay.

Since the optimum salinity for setting ranges from approximately 15 to 25 per mille it follows that either an increase or decrease may favor attachment of the larvae but such a change is not necessary for this reaction. The author

has observed, during dry seasons, heavy setting in areas that are normally of very low salinity. The reverse is also true in regions of high salinity such as Long Island Sound after periods of excess river discharge. Here the high salinity apparently increases also the length of time for complete larval development which required 30 days or twice the normal time during the dry summer of 1929.

Since the salinity in many setting regions fluctuates considerably according to river discharge, wind and tidal conditions, it is evident that significant data concerning this factor must be obtained at the actual stage of tide, depth, and time that setting is in progress.

PHYSICO-CHEMICAL CONDITIONS INVOLVED IN SETTING REACTION

After determining the essential features of the setting process a series of experiments were conducted for the purpose of determining which environmental factor was most important in initiating this developmental change. The more outstanding factors such as temperature, salinity, hydrogen-ion concentration, etc., were tested under laboratory conditions and covered a range that was slightly in excess of that found under natural conditions. The results of this series which were carried out during the summer of 1929 and repeated in 1930 are given in Table 3.

In this series no evidence of setting was found either in the controls or experimental solutions. Numerous experiments were also made involving

TABLE 3. Effect of temperature, salinity, etc., on setting.

Factor	Range	Other conditions	Effect on setting
Temperature	15° – 30° C	Salinity 15, pH 8.0	Negative
	15° – 30° C	Salinity 20, pH 8.0	do
	15° – 30° C	Salinity 25, pH 8.0	do
Salinity	5 – 35 per mille	Temp. 20° C, pH 8.0	do
	5 – 35 per mille	Temp. 25° C, pH 8.0	do
	5 – 35 per mille	Temp. 30° C, pH 8.0	do
Hydrogen-ion concentration	7.0 – 9.0 pH	Salinity 15, t. 25° C	do
	7.0 – 9.0 pH	Salinity 20, t. 25° C	do
	7.0 – 9.0 pH	Salinity 25, t. 25° C	do
Oxygen content	50 – 100% sat.	Salinity 20, pH 8.0	do
Carbon-dioxide tension	0.003 – 0.0003 mg.CO_2 per c c	Salinity 20, t. 25° C (pH 7.0 – 8.0)	do
Water pressure	0.1 – 6.5 feet	Salinity 20, pH 8.0, temp. 25° C	do
Current velocity	0 – 1.5 ft. per sec.	Salinity 20, pH 8.0, temp. 25° C	do

sudden changes in temperature, salinity, etc., which likewise proved ineffective in stimulating setting.

It was apparent, however, from previous observations that the solution to the problem was to be found in the mixing of river water and sea water. The intensity of setting during periods when such mixing is most pronounced might be attributed either to some physico-chemical change taking place at such times or to some particular element that was contributed by the river water. An enormous mass of soluble salts is annually carried to the sea by river waters but their composition in respect to the relative proportion of the various elements is very different from that of the ocean. At present little is known of the chemical changes that take place in ocean water or of the reason for the difference in sea salts and those received from streams. Clarke (1924) states that in chemical character fresh and salt water are opposites, as shown, for example, by the fact that in ocean water $Cl > SO_4 > CO_3$, while in average river water, $CO > SO_4 > Cl$. This likewise holds for the bases which in the first case show that Na> Mg> Ca, while in river water the order is completely reversed and Ca> Mg> Na. The same is true for potassium and some of the heavy metals such as copper, iron, zinc, etc. which are more abundant in river water than in the ocean. It is evident, therefore, that complex chemical reactions are constantly taking place in the inshore waters and particularly those which receive considerable drainage from the land.

The oyster larvae attached in Milford Harbor during the low-water stage of the tide. At such times the interaction of fresh water and sea water is apparently most pronounced which suggested the advisability of testing first the effect of the neutral salts on setting. Several series of experiments were conducted in which the amount and proportion of the cations and anions of the neutral salts were varied.

Solutions of the chlorides, sulphates, carbonates, and nitrates of sodium, potassium, magnesium, and calcium were prepared in glass distilled water in such concentration that the metal and radical ions could readily be added in known parts per million. The concentrations used were for the most part comparable with those occurring in river water and varied from 0.1 to over 100 parts per million in respect to their content of each particular anion or cation. The salinity of river waters of the Atlantic slope as shown by Clarke (1924) varies from 57 to 155 parts per million while that of the ocean averages about 35,000 parts per million. One milliliter portions of each neutral salt solution were added to 9 milliliters of sea water containing from 10 to 20 full grown larvae. Natural sea water of three different salinities (15, 20 and 25 per mille) was used in which the pH ranged from 7.9 to 8.1 and the temperature from 24° to 27° C.

This entire series of experiments showed that the cations Na, K, Mg, and Ca and the anions Cl, SO_4 CO_3, and NO_3 would not stimulate setting of the

oyster larva. Further experiments involving various combinations of the neutral salts and artificial freshwater solutions containing these salts in concentrations comparable with river water were likewise ineffective in inducing setting.

River water brings down small amounts of many heavy metals which may be either in solution, or in suspension as absorbed or adsorbed elements on the fine particles of clay, sediment, or plant and animal debris. The rivers of the Atlantic slope are largely of the carbonate type and by virtue of their high carbonic acid content are active agencies in dissolving and carrying in solution the metallic elements in the rocks. At the mouths of the rivers these elements are usually present in greatest concentration particularly during the low water stage of the tide. Experiments were conducted to test the effect of each metal ion on setting. The oyster larvae were exposed to ten different heavy metals in concentrations varying from traces to toxic amounts, which were added to solutions having salinities ranging from 15 to 25 per mille. The tests were made either by dipping for short intervals small pieces of the pure metal into the seawater containing the larvae or by the addition of solutions containing definite amounts of the carbonates, sulphates, and chlorides of aluminum, manganese, iron, nickle, copper, zinc, silver, tin, barium, and lead.

The metal carbonates are insoluble in water and particularly seawater so it was necessary to acidify solutions of these salts with HC1 and then neutralize them just before using. In this manner a colloidal or finely divided precipitate of the metal was obtained which could be taken readily into the digestive tract of the larva. In fact most of the heavy metals are quickly precipitated in an alkaline medium such as seawater and only very minute amounts remain in solution in equilibrium with the dissolved salts. In several of the experiments the hydrogen-ion concentration was varied by means of CO_2 from a pH of 8.2 to 7.0 in order to increase any possible effect which the free metal ions might have on setting. The results obtained with the heavy metal series are summarized in Table 4.

In two of the preliminary tests with heavy metals setting was observed after the addition of small amounts of ferrous chloride and barium chloride, but this was found to be due to traces of copper in these salts and did not occur later when copper free chemicals were used. The only element in the heavy metal series that produced a positive setting reaction was copper in the form of a pure metal or as a dissolved salt. This heavy metal was found to be effective and initiated almost immediately the setting process in concentrations as low as 1 part copper to 25 million or 50 million parts of sea water. In the controls the larvae lived for a week or two but eventually died after failing to set or metamorphose. However, if copper stimulation was applied during this period the larvae would set and develop into healthy spat.

TABLE 4. Effect of heavy metals on setting.[1]

Element	Type of substances used	Range of concentration metal content p.p.m.	Effect on setting
Aluminum	$Al_2(SO_4)_3$	1 to 20	Negative
	$AlCl_3$	do	do
	Metallic	Immersion 1 min. – 6 hrs.	do
Manganese	$MnCO_3$	1 to 50	Negative
	$MnSO_4$	do	do
	$MnCl_2$	do	do
Iron	$Fe_2(SO_4)_3$	0.1 to 10	Negative[2]
	$FeCl_3$	0.05 to 20	do
	$FeCO_3$	0.1 to 10	do
	$FeSO_4$	do	do
	$FeCl_2$	do	do
	Metallic	Immersion 1 min. – 24 hrs.	do
Nickel	$NiCO_3$	0.05 to 5	Negative
	$NiSO_4$	do	do
	$NiCl_2$	do	do
	Metallic	Immersion 1 min. – 1 hr.	do
Copper	$CuCO_3Cu(OH)_2$	0.05 to 1	Positive[3]
	$CuSO_4$	do	do
	$CuCl_2$	do	do
	Cu_2CO_3	do	do
	$CuOH$	do	do
	Cu_2Cl_2	Metallic 10 sec. – 5 min.	do
Zinc	$ZnCO_3$	0.5 to 5	Negative
	$ZnSO_4$	do	do
	$ZnCl_2$	do	do
	Metallic	Immersion 1 min. – 6 hrs.	do
Silver	$AgCl$	0.05 to 1	Negative
	$AgNO_3$	do	do
	Metallic	Immersion 1 – 24 hrs.	do
Tin	$SnCl_4$	0.5 to 5	Negative
	$SnCl_2$	do	do
	$SnSO_4$	do	do
	Metallic	Immersion 1 – 24 hrs.	do
Barium	$BaCO_3$	0.05 to 5	Negative
	$BaSO_4$	do	do
	$BaCl_2$	do	do
Lead	$PbCO_3$	0.1 to 10	Negative
	$PbSO_4$	do	do
	$PbCl_2$	do	do
	Metallic	Immersion 1 – 24 hrs.	do

1. Solutions of 3 different salinities (15, 20 and 25 per mille) and 2 different hydrogen-ion concentrations (pH 7.5 and 8.0) were used in these experiments.
2. In higher concentrations larvae are surrounded and smothered by colloidal precipitate.
3. Cu toxic in concentrations above 0.5 p.p.m. Lower concentrations of all copper salts stimulate setting.

In several experiments various alloys of copper containing zinc, tin, lead, and iron were used successfully in stimulating setting. It was observed that the copper was noticeably less apt to produce toxic effects in the presence of

small amounts of other metals than when used as a pure substance. In river water the presence of many metals in traces or small amounts may likewise be a beneficial condition as it would tend to limit the action of certain elements on plant and animal tissues. In this connection Brenchley (1927) presents evidence in his monograph on "Inorganic plant poisons and stimulants" showing that the toxic action of certain metal ions such as copper is decreased in presence of other elements of this group. The manner in which this is effected is perhaps comparable with the ion antagonism observed in the neutral salts except that much smaller amounts of the elements are involved.

In concluding the analysis of numerous physical and chemical factors in respect to setting it has been found up to the present time that copper is the specific element that will induce this reaction and that its presence in the water in minute amount is necessary for attachment of the oyster larva. Though river discharge is responsible for many fluctuations in the chemical composition of the water over the oyster beds, its continual contribution of minute amounts of copper is the factor of primary importance in setting of the oyster. The effect of copper on larva and spat will first be reviewed in greater detail before proceeding with the analyses of river and brackish water for copper which are presented later.

DETAILED PHYSIOLOGICAL OBSERVATIONS ON THE EFFECT OF COPPER

Copper, like many other heavy metals, has been found to have a poisonous effect upon living material and possesses the power of oligodynamic action wherein it is seen that infinitesimal amounts produce results that are apparently out of all proportion to the quantities involved. On the other hand, it has been shown in the previous studies that copper is not always toxic but may have a very beneficial and stimulating effect upon an organism when it is present in such low concentrations as are found under natural conditions. Both the toxic and beneficial effects of copper on oyster larva are of importance. There are certain regions in which trade waste pollution has increased the copper content of the water so greatly as to render it toxic to oyster larvae and spat. Waste of an alkaline character may likewise be detrimental by causing precipitation of normal amounts of copper from river waters before they reach the oyster beds. Consequently, experiments were carried out to determine (1) the reaction of dissolved copper salts in sea water, (2) the effect of various concentrations of copper on oyster larvae, and (3) the nature of the process by which copper is assimilated by the larvae.

As stated previously many elements including copper are more abundant in river water than in the ocean. It is evident, therefore, that in the brackish water areas some elements are precipitated out and deposited with the sedi-

ments, some remain in solution, while others are incorporated in the tissues of plants and animals.

When fresh water containing a dissolved copper salt mixes with brackish or sea water, the metal is precipitated in the form of a metacolloid by the action, apparently, of a higher concentration of electrolytes in an alkaline medium. Exact analysis of this complex process was not possible with the chemical equipment at hand. However, by using greater amounts of copper it was possible to obtain in sea water a flocculent colloidal precipitate of light blue-green color. Its formation is presumably analagous to that described by Clarke (1924) for manganese which, under similar conditions, is oxidized by the dissolved oxygen of the sea water. Manganese nodules found on the sea bottom contain nearly 1 per cent copper oxide. Our analysis of the copper colloid indicates that it is copper oxychloride, $CuCl_2.2CuO.4H_2O$. It is insoluble in fresh water and sea water and resembles in many respects a secondary ore of copper known as atacamite, $Cu_2Cl(OH)_3$, which is formed under natural conditions when metallic copper is acted upon by saline solutions.

In the littoral zone the surface and underground waters are most actively giving up their load of dissolved and suspended materials and here we find the maximum precipitation of this colloidal copper salt. This precipitate is light and flocculent and may be distributed for considerable distances before finally settling to the bottom. The formation of this copper colloid takes place very rapidly during the mixing of fresh and salt water, and this is undoubtedly the chemical state in which this metal is taken in by the oyster larvae under natural conditions.

In the studies of the effect of various concentrations of copper on larvae, the sulphate, $CuSO_4\ 5H_2O$, the chloride, $CuCl_2$, and the colloidal sea water salt (copper oxychloride) were used. Standard solutions of copper sulphate and chloride were made up in distilled water in a concentration of 0.0001 gram of copper per milliliter while the colloidal copper was obtained through the precipitation of known amounts of these salts in an excess of sea water. When the solutions containing dissolved copper were added to sea water in such small amounts as to have less than 0.5 mg. of copper present per liter it was found that virtually all the copper was precipitated, while with greater concentrations there still remained an excess of free metal ions in the solutions. Consequently, the larvae in some cases were subjected to the action of both dissolved and combined copper while in the lower concentrations the effect was largely due to ingestion of suspended particles of colloidal copper.

The solutions were made up in Syracuse glasses in 10 c.c. portions into which several oyster larvae were placed and kept under microscopic observation. In the controls the larvae swam about or lay on the bottom, keeping the velum protruded most of the time beyond the edges of the shell with the

powerful cilia beating regularly. Reactions of the larvae to the presence of chemicals may be seen, through change in the rate of beat of the velar cilia, by retraction of the velum, by protrusion of the foot, or by closure of the shell. When the larvae were placed in highly toxic solutions containing as much as 20 mg. of copper per liter, they swam about for a few minutes and then suddenly retracted the velum, closed the valves tightly and settled to the bottom. During this short swimming or feeding period which lasted for an interval of from 3 to 5 minutes, the larvae received a lethal dose of copper from which they were unable to recover even if transferred immediately to copper free sea water.

Exposure to copper concentrations ranging from 3 to 20 milligrams per liter for periods of 3 to 5 minutes was always fatal to the larvae and resulted in a gradual disintegration of the tissues through the cytolytic action of the copper. It was found that in these higher concentrations the larvae were subjected to both free and combined copper and to a slight increase in hydrogen-ion concentration when copper sulphate or chloride solutions were added. To eliminate free copper ions and hydrogen-ion concentrations as toxic factors, the copper was added in equivalent amounts in the form of the colloidal precipitate of copper. A similar reaction of the larvae was obtained in these experiments in which it could be seen that finely divided particles of the copper colloid were carried into the digestive tract in sufficient amount to prove fatal, before the larvae were sensitive to its presence.

Within the digestive tract it is probable that the metal colloid was dissolved through increased hydrogen-ion concentration so that the copper ions were released and passed into the tissues of the larva. This interpretation receives further support from the fact that the larva reacted to the presence of copper after a latent period of 3 to 5 minutes which is the length of time required for particles of carmine to reach this portion of the digestive tract. In subsequent experiments where nontoxic amounts of copper were used the duration of the latent period was the same and showed an average interval of 4 minutes and 20 seconds from the time of copper addition to retraction of the velum or protrusion of the foot.

Copper concentrations ranging from 0.8 to 2.5 mg. per liter likewise proved toxic if the larvae remained in them long enough to receive several consecutive doses of the copper salt. On the other hand, these concentrations induced setting and produced normal spat formation if the larvae were immediately transferred to copper free water after the first ingestion of copper particles.

Solutions containing 0.5 mg. or less of copper per liter are nontoxic to the larvae even when they were exposed continuously to this concentration of the metal. When both mature and immature larvae are placed in these low copper concentrations, the former began crawling after a latent period of 4

to 5 minutes and eventually set, while the latter swam intermittently at first, and continuously later after most of the copper had apparently been extracted from the water.

The oyster larvae were similar to other animals and plants in that they were able to ingest and assimilate many inorganic elements such as zinc, iron, copper, etc., without deleterious effects when these substances were present in infinitesimal amounts. Under natural conditions, the larvae were subjected to marked periodical changes in the concentration of these elements during each tidal cycle. They tended to react more definitely when these elements were present in greatest amount. It is important that several metals are in solution or suspension at such times as this limits the toxicity of any particular one according to Brenchley (1927). It was probable that this condition likewise held in respect to the effect of copper on oyster larvae as indicated by the previous experiments with alloys of this metal.

Traces of copper were found in the sea water at all times but in order for this element to have a sufficiently stimulating effect to induce setting, it must be present in concentrations of the order of 0.05 to 0.6 mg. per liter. For example, in the controls the larvae received frequent changes of water collected at the time of high tide which contained only traces of copper, but in these solutions setting never occurred though the mature larvae would live in them for 10 to 14 days. On the other hand, if a piece of metallic copper was dipped in these solutions for a few seconds or if 0.001 to 0.005 mg. of a copper salt was added to a 10 cubic centimeter portion, the larvae were stimulated sufficiently to undergo the setting process.

One interesting fact brought out by these experiments and the field studies is that the stimulating power of the copper increased with concentration and was most potent when these concentrations approach toxic values (Fig. 14). The concentrations of a stimulative and beneficial nature are intermediate between those which are toxic and those which are so low as to produce no evident effect. Similar results have been obtained on plants and other animals with copper and various inorganic poisons as reported by Mathews (1905), Cook (1926), Brenchley (1927), True (1930), and LeVan (1930).

The results obtained in the present studies with copper may be interpreted as due to differences in the permeability of the ions in relation to their concentration. So long as the solution of a copper salt is dilute enough, the absorption layer of the exposed cells, acting as a semipermeable membrane, is able to keep the copper out of the organism and check its toxicity. As soon, however, as a certain limit is reached, it is probable that the copper exercises a corrosive influence upon the cell membranes whereby their permeability is increased so that they are no longer able efficiently to resist the entry of the poison. The copper ions are then able to permeate the cell protoplasm and affect its vital processes. When the copper is present in greater

amount the toxic action is dominant and many cellular functions may be seriously impaired. On the other hand, with a slightly lesser concentration of the metal ions, differentiation of action may set in so as to increase cellular metabolism through the stimulation of some processes or hindrance of others.

The studies of the catalytic action of copper in the oxidation of glutathione by Elliott (1930), and Meldrum and Dixon (1930) show how this element in exceedingly small amounts may have a marked effect in increasing cellular respiration. With higher concentrations, Voegtlin, Johnson, and Dyer (1925) have shown how the toxic effect of copper may be due to a disturbance of the glutathione equilibrium in the cell.

When the larvae were exposed to toxic amounts of copper, they did not die immediately but gradually disintegrated over a period of several days. First, the velar cilia were cast off en masse or the entire velum amputated from the body after which the remaining tissues gradually shrunk and broke apart in small pieces. In a few experiments using slightly toxic concentrations of copper (0.8-1.5 mg. p.l.) the effects of the copper were found to be rather intermediate between those which were distinctly injurious and those which were stimulating, as shown in Figure 11 A, B, and D. The copper produced setting in the usual manner which was followed by the formation of a spat shell, but the transformation and development of the internal organs of the spat were retarded and abnormal so that death of the organism occurred after a few days.

In the case shown in Figure 11 *B* the larval foot persisted and continued to grow after setting, while the gills failed to develop and gradually disintegrated. In Figure 11 *A* a larva is shown that has shed the large velar cilia, which is apparently but a slight injury as this was found to occur in several instances previous to normal setting and development of healthy spat. The slow but progressive toxic action of the copper was found in the cases shown in Figure 11 *B* and *D* in which growth of the gills and spat organs continued for a short time but was followed later by shrinkage and cytolysis of the tissues.

It is evident that it is exceedingly difficult to separate definitely the reactions of copper as a toxic substance or stimulant, as both produce differential changes in the larval and spat tissues. When these changes are of a progressive nature we regard copper as a stimulant, but on the other hand when they are regressive and eventually cause death we consider copper as a poison. The quantitative difference between the two as approximated by copper colloid feeding experiments is of the order of magnitude of 0.0005 to 0.002 of a milligram of copper. The production of injurious or stimulating effects depends primarily upon a sudden concentration of copper within the larva rather than upon the continual ingestion of traces of the metal over a longer period of time.

The studies of Huxley (1922) are of interest in this connection as they likewise show that the metamorphosis and dedifferentiation of Echinus larvae are initiated through the action of toxic agencies. He regards dedifferentiation as a process that is initiated through the destructive action of a toxic stimulant, as a result of which certain cellular structures are incapacitated

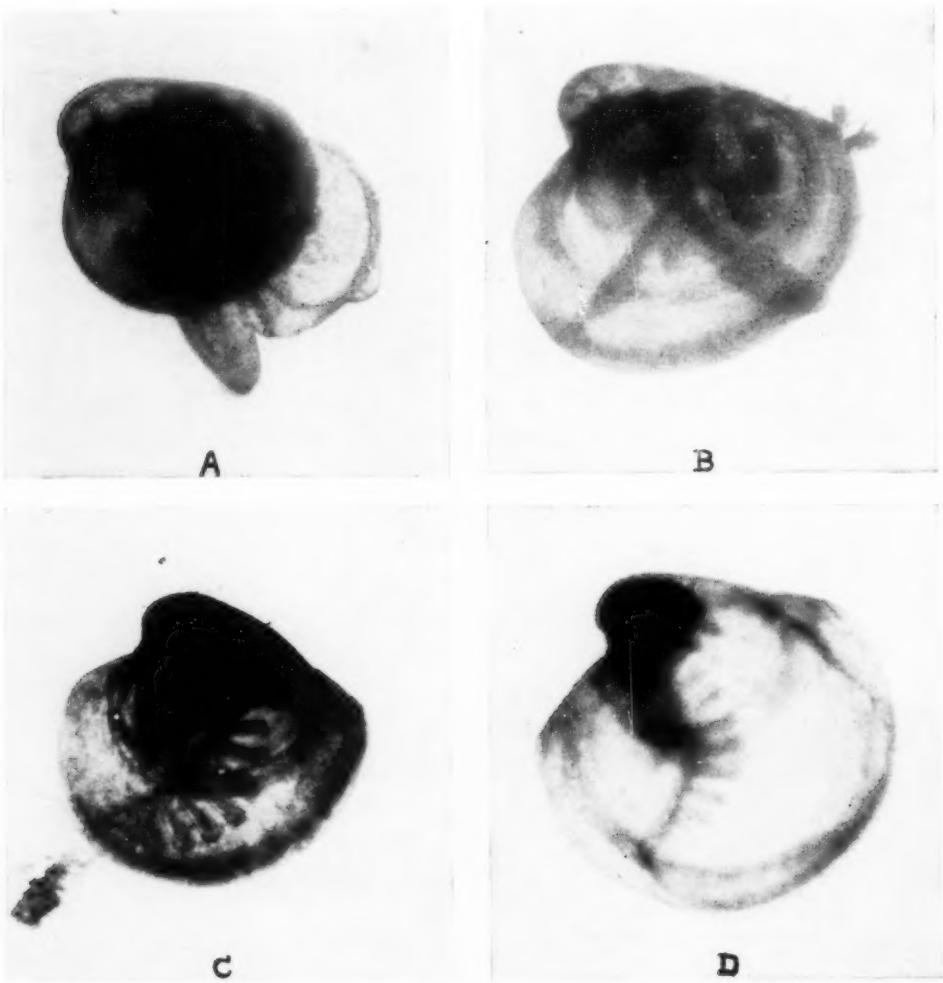

FIG. 11. Effects of toxic amounts of copper on oyster larva and spat. A, loss of velar cilia; B, incomplete metamorphosis; persistence of foot, retarded gill development; C, normal spat, 48 hours after setting; D, abnormal spat, 9 days old.

while others are benefited. However, in the development and differentiation of the oyster larva it is more logical to interpret the action of normal amounts of copper as beneficial, as this element undoubtedly increases the functional capacity and growth of the cells and tissues.

During the two weeks that larval development is in progress the organism receives stimulating amounts of copper for a short period during each tidal

cycle. At the same time, other elements such as iron, zinc, lead, etc., are also ingested in minute amounts which as mentioned previously decreases the toxic action of the copper ions and probably facilitates their passage into the tissues. This periodical stimulation is apparently an important factor in larval development, and failure to duplicate this essential environmental condition may be responsible for the repeated failures in the artificial culture of marine forms. For example, in 1929 when the small supply of heavy metals by river discharge was greatly reduced as a result of the dry weather, the complete development of the oyster larvae required over 30 days or twice the normal time.

In summarizing these experiments, it is concluded that the stimulus for setting of the oyster larva is obtained through the absorption and specific action of copper ions within the organism, rather than to some physical or chemical change in the surrounding medium. The copper is extracted from the water by the larva in the form of a suspended, colloidal copper salt, or possibly in some cases as an adsorbed cation on clay particles or sediment. It is transported by ciliary action into the mouth and esophagus and in a few minutes reaches the stomach and intestine. Here dissociation of the copper compound and release of copper ions is most likely to occur because of the greater hydrogen-ion concentration. The assumption of a greater hydrogen-ion concentration in the gut of the larva is based on the work of Yonge (1926) in which it is shown that the average pH of the stomach and style of the adult is 5.5 and 5.2 and is acid, while the surrounding sea water has a pH of 8.0 and is alkaline.

After the mature larva has ingested copper in nontoxic amounts, the byssal gland is stimulated and the byssus is secreted from the bottom of the foot while this organ is protruded beyond the edges of the shell. At the same time, the beating of the velar cilia is greatly increased and the larva travels through the water rapidly in search of an object for attachment. The setting reaction begins after a latent period having an average duration of 4 minutes and 20 seconds which approximates the interval required for passage of the copper into the digestive tract and its assimilation therefrom.

The copper was most effective in initiating setting when the amount present varied from 0.05 to 0.6 milligram per liter. Within this range of concentration the number of larvae responding to this stimulus was directly proportional to the amount of copper present. On the other hand, copper was highly toxic to the larva when they were exposed continuously to concentrations exceeding 0.8 milligram per liter while the presence of this element in traces of less than 0.02 milligram per liter had no evident effect upon them, and would not induce setting.

RELATION OF COPPER TO METAMORPHOSIS

In the transformation of the oyster larva into a spat, copper occupies an equally important position to that observed in relation to setting. After the larva has cemented itself to some submerged object; the reorganization of the tissues and development of adult organs proceeds at a rapid rate. A few of the most significant changes which occur at this time are: (1) disappearance of the pigment spots; (2) atrophy of the velum and foot; (3) rapid growth of the gills and mantle; (4) development of the posterior adductor as primary shell muscle; (5) increase in heart action and blood circulation; and (6) appearance of leucocytes in the blood stream.

In the larva the two pigment spots of dark green color are rather outstanding structures which were used in the setting experiments as an index of larval maturity. One pigment spot is found on each side of the body as shown in Figure 4 and consists of a mass of densely packed cells, containing numerous green colored granules. Each spot lies just outside of the digestive diverticula or so-called "liver" in what appears to be an endothelial sac or enlarged portion of the dorsal blood vessel.

When setting is in progress, the pigmented cells in each spot gradually enlarge, separate, and one or two break away and are carried forward by the blood stream into the mantle. After attachment, the pigment spot breaks apart more rapidly; and all the cells become distributed in a short time into the circulatory system, as the process of transformation of the larva is in full swing. Each pigment spot consists of approximately 300 cells which after separation, resemble in many respects the leucocytes of the spat and adult oyster. The breaking up of the pigment spots is an important and necessary preliminary step to metamorphosis, and requires copper as the initial stimulus. This conclusion was derived from the following experiments.

A total of 2,781 larvae induced to set by minimum doses of copper were collected on numerous glass slides and Syracuse glasses. Immediately after attachment three batches of larvae were transferred to separate glass aquaria containing high tide water in which copper was present in only slight traces, while a fourth was placed in the harbor under natural conditions. In 24 hours none of the larvae in the aquaria had undergone even partial metamorphosis and the pigment spots showed but slight evidence of breaking apart. However, those placed under natural conditions showed in 18 hours complete metamorphosis, loss of pigment spots and the first development of spat organs and shell.

In order to test the effect of copper on metamorphosis several batches of the attached larvae were placed in high tide water to which copper had been added in amounts varying from 0.1 to 0.5 milligram per liter. In these solutions metamorphosis occurred and the dissolution of the pigment spots was greatly accelerated. Many of these larvae after exposure to the copper for

an interval of 30 minutes to 2 hours, were returned to the aquaria where they developed rapidly into spat and continued to grow in the high tide water which contained insufficient copper for metamorphosis.

In the controls the attached larvae were given frequent changes of high tide water but failed to metamorphose and died after a period of 10 to 14 days. Up to about 9 days, these larvae could be induced to undergo metamorphosis if they were fed the colloidal precipitate of copper or if they were placed in low tide water containing from 0.25 to 0.6 milligram of copper per liter.

Therefore, it is evident that under natural conditions the higher copper content of the water during low tide periods is a beneficial and necessary stimulus not only to setting, but also to the subsequent metamorphosis of the larva into a spat. Though many morphological changes occur in the transformation of the larva into an adult oyster, those which appear to be of greatest biological significance are concerned with the breaking up of the two pigment spots after ingestion of minute amounts of copper. Metamorphosis follows the release into the circulatory system of the numerous cells, containing a blue-green pigment, which compose each pigment spot. According to Galtsoff and Whipple (1930) there is a definite relation between the green pigment in adult oysters and the amount of copper present. These authors found that the intensity in green color was in proportion to the copper content of the oyster and that histologically the copper is located in the green leucocytes.

Pigments formed by the combination of certain heavy metals with various porphyrins are important respiratory catalysts and play an essential rôle in cellular oxidations (see Anson and Mirksy, 1930). In the blood of the oyster and certain invertebrates the copper pigment which carries oxygen appears to be a copper porphyrin compound similar to iron prophyrin which is the principal heme pigment of vertebrates.

Therefore, in the release of pigmented cells into the blood stream of the oyster larva after attachment there comes into play an important mechanism for increasing the respiratory processes of this mollusk. This appears to be the initial use of pigmented cells in the blood stream of the oyster and is undoubtedly of fundamental importance not only in its immediate metamorphosis and growth but in its past development and evolution.

COPPER CONTENT OF BRACKISH WATER

Since the previous studies showed that copper is the primary controlling factor in setting, the next step had been to determine (1) to what extent copper is normally present in the environment of the oyster; and, (2) the relation between the copper content of the water and the time, place, and intensity of the setting reaction.

During the period from July 15, to August 7, 1930, the setting of millions of oyster larvae was in progress in Milford Harbor, Conn., so that it was possible to make copper analyses of the water at the time that attachment of the larvae was actually occurring in greatest intensity. The first series of samples were taken during the low water stage of the tide at which time the larvae were most abundant and were going through the setting process. Though this fact was established by the author in 1925 and 1926, it was carefully checked again by plankton and spat collection experiments during the summer of 1930 in connection with the copper analyses.

Setting occurs here chiefly in the surface layer of water at low tide and is heaviest in a zone extending vertically from a point 1 foot below low water level to a point 1 foot above. In Table 5 an analysis is given of the copper content, salinity, temperature, and hydrogen-ion concentration of the surface layer of water at the time of low water when setting was in progress.

TABLE 5. Copper content of the water at low tide.

Date	Copper mg. per liter	Salinity per mille	Temperature °C	H-ion conc. pH
July 15	0.2	27.51	24.0	7.9
17	0.1	27.23	24.8	7.8
18	0.2	27.03	26.2	8.0
19	0.4	28.00	26.5	7.9
21	0.2	27.64	25.5	8.0
24	0.5	27.76	27.0	8.1
26	0.3	28.05	28.8	8.2
29	0.4	27.95	26.5	8.2
Aug. 1	0.1	27.83	27.0	8.0
5	0.2	27.97	25.9	8.1
7	0.2	27.99	27.6	8.0
Average	0.25	27.72	26.3	8.0

It is evident from the previous analyses that copper is present in the sea water in measurable amounts during the low tide periods so that chemical stimulation of the larvae by this element was possible under the existing natural conditions. Similarly water samples from New Haven, Black Rock, and Southport Harbors that were collected at the same stage of tide were found to have a copper content ranging from 0.2 to 0.6 milligram per liter.

In inshore areas of this type the copper content varies according to the stage of tide. Analyses covering several complete tidal cycles, as presented in Figures 12 and 13, show the highest concentration of this element during low water periods or, in other words, when the chemical effect of river discharge is greatest. Previous experiments indicated that river water contained copper as it induced setting, and lost this stimulating power when it was treated for removal of this element. On the other hand, water coming in from the sea is apparently not a significant source of this element as the

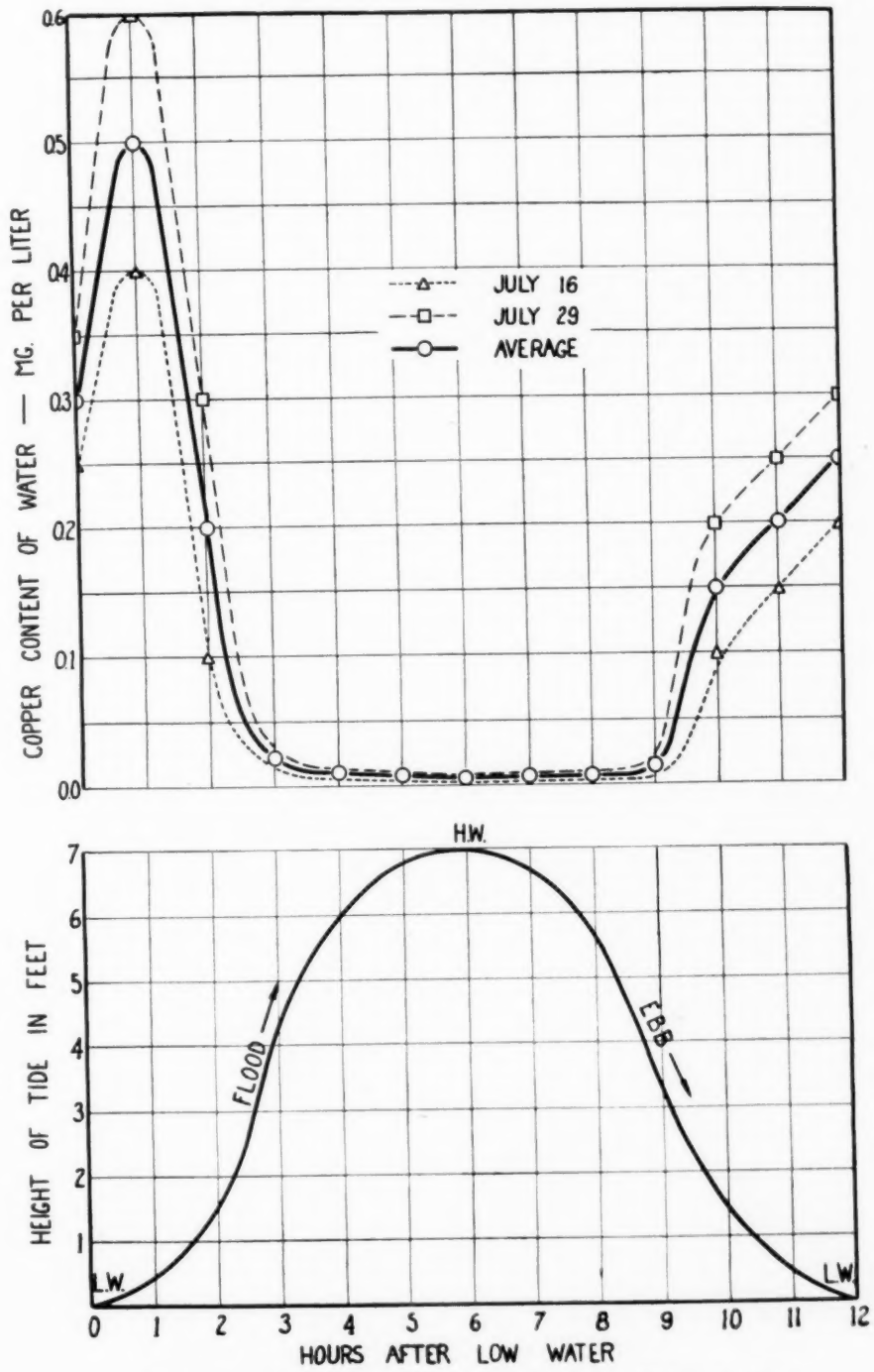

FIG. 12. Fluctuation in copper content during tidal cycle (low water to low water) on July 16, and July 29, 1930, Milford Harbor.

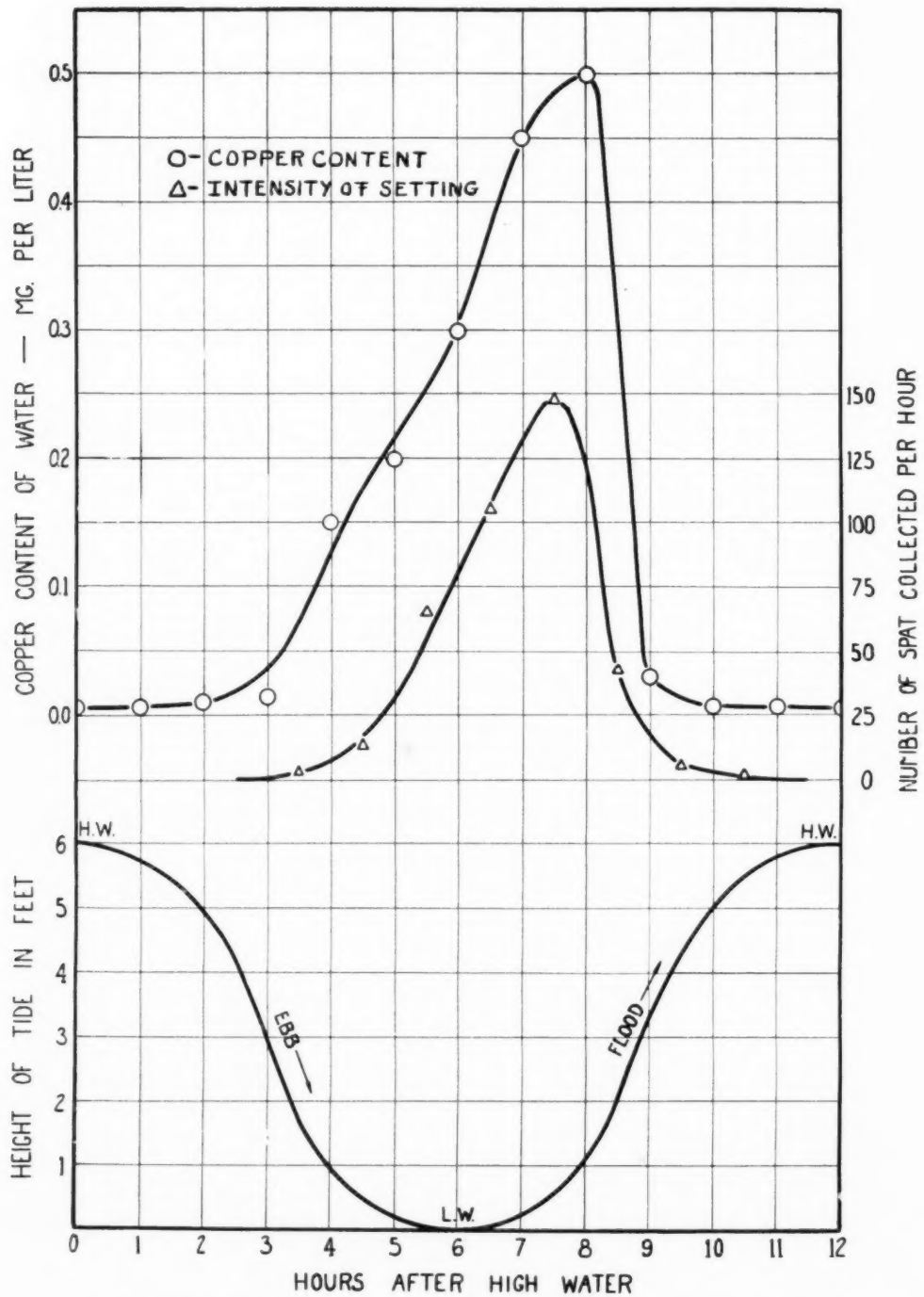

FIG. 13. Variation in the intensity of setting and copper content of the water during tidal cycle (high water to high water) on July 22, 1930, Milford Harbor.

copper content of the harbor waters is lowest at time of high tide. As we proceed from the sea to the mouths of the rivers there is a steady increase in the copper concentration of the water, which indicates that river water is probably the chief agency supplying this element to inshore coastal areas.

VARIATIONS IN COPPER CONTENT IN RELATION TO SETTING

Since the setting of the larvae occurred chiefly during the low tide periods, it was desirable to know if there was a definite relation between the time and intensity of this reaction and the copper content of the water. Consequently, on July 16, 22, 29, and August 4, hourly samples were taken for copper analysis, during four complete tidal cycles. In every case the water samples were collected always at the same definite point or level, the location of which coincided with the heavy setting zone. The results of these observations are presented in Tables 6 and 7 and Figures 12 and 13.

The series of observations shown in Figure 12 were made on July 16 and July 29 and covered tidal periods extending from low water to high water and back again.

The series made on July 22 and August 4 show similar results but were made during tidal periods extending from high water to low water and return. Conditions in Milford Harbor were unusually favorable and constant for this work as there were no heavy rains or storms during this period to disturb the regular inflow and mixing of fresh and salt water in this area.

On July 21, the examination of plankton samples and spat collectors showed that full grown oyster larvae were abundant in the water and were setting in large numbers during each tidal cycle. Consequently, on the following day an experiment was carried out to determine the intensity of setting during each hour in relation to the copper content of the water and stage of tide. For this purpose the standard partition collectors for oyster seed, shown in Figure 2, were used, 12 of which were planted under uniform conditions at the time of high water and removed singly every hour thereafter to ascertain the number of spat which they had gathered during each interval. The cement coated partitions were seasoned in sea water for several days before planting and after obtaining a set were kept in a large tank until the spat were large enough for counting. The difference in the number of spat obtained per collector from hour to hour indicates the relative intensity of setting during each period and is shown in the following Table 8 and Figure 14 together with data on the copper content of the water and stage of tide.

Since each spat represents a completed and successful setting reaction, it is obvious from the examination of Table 8 that a greater percentage of oyster larvae participated in this reaction during the tidal stages when copper

TABLE 6. Variation in copper content of water during tidal cycle on July 16, and July 29, 1931.

Tide	Hours after low water	Depth of Sample in feet	Copper mg. p.l. July 16	Copper mg. p.l. July 29	Average cu. content
Low water..	0	1.0	0.25	0.35	0.30
Flood.....	1	1.5	0.40	0.60	0.50
do.....	2	2.5	0.10	0.30	0.20
do.....	3	5.3	0.015	0.025	0.020
do.....	4	7.0	0.010	0.010	0.010
do.....	5	7.8	0.008	0.012	0.010
High water..	6	8.0	0.002	0.008	0.005
Ebb.....	7	7.6	0.000	0.004	0.002
do.....	8	6.5	0.000	0.012	0.006
do.....	9	4.2	0.005	0.015	0.010
do.....	10	2.5	0.10	0.20	0.15
do.....	11	1.4	0.15	0.25	0.20
Low water..	12	1.0	0.20	0.30	0.25

TABLE 7. Variation in copper content of water during tidal cycle on July 22, and August 4, 1931.

Tide	Hours after high water	Depth of sample in feet	Copper mg. p.l. July 22	Copper mg. p.l. August 4	Average cu. content
High water..	0	7.0	0.006	0.004	0.005
Ebb.....	1	6.7	0.004	0.002	0.003
do.....	2	6.0	0.010	0.006	0.008
do.....	3	4.0	0.015	0.005	0.010
do.....	4	1.9	0.150	0.050	0.100
do.....	5	1.2	0.200	0.100	0.150
Low water..	6	1.0	0.300	0.100	0.200
Flood.....	7	1.3	0.450	0.250	0.350
do.....	8	2.0	0.500	0.400	0.450
do.....	9	4.3	0.030	0.020	0.025
do.....	10	6.0	0.008	0.002	0.005
do.....	11	6.8	0.008	0.004	0.006
High water..	12	7.0	0.006	0.000	0.003

was present in appreciable amount. The number of larvae which cemented themselves to the collectors varied from hour to hour in direct proportion to the copper content of the water as shown graphically in Figures 13 and 14.

Collectors which were in the water for the complete tidal cycle on July 22, showed a concentration of approximately 380 spat per collector. Over 97.5 per cent of these spat had become attached when the copper concentration ranged from 0.15 to 0.50 milligrams per liter, which was approximately 40 per cent of the total time that the collectors were exposed.

In Milford Harbor, the water of highest copper content is found in the surface layer shortly after the time of low water, while in other regions this relationship may vary according to the existing hydrographical and tidal conditions and thus produce differences in the distribution of oysters and heavy setting areas. The sudden rise in the copper content of the water on the first of flood tide as shown in Figure 13 is due to certain hydrographical features

of the harbor and their effect on the circulation of the water at different tidal stages. Above the sampling station, the fresh water flowing into the harbor comes largely from the Wepawaug River while below the station a further supply is obtained from the Indian River.

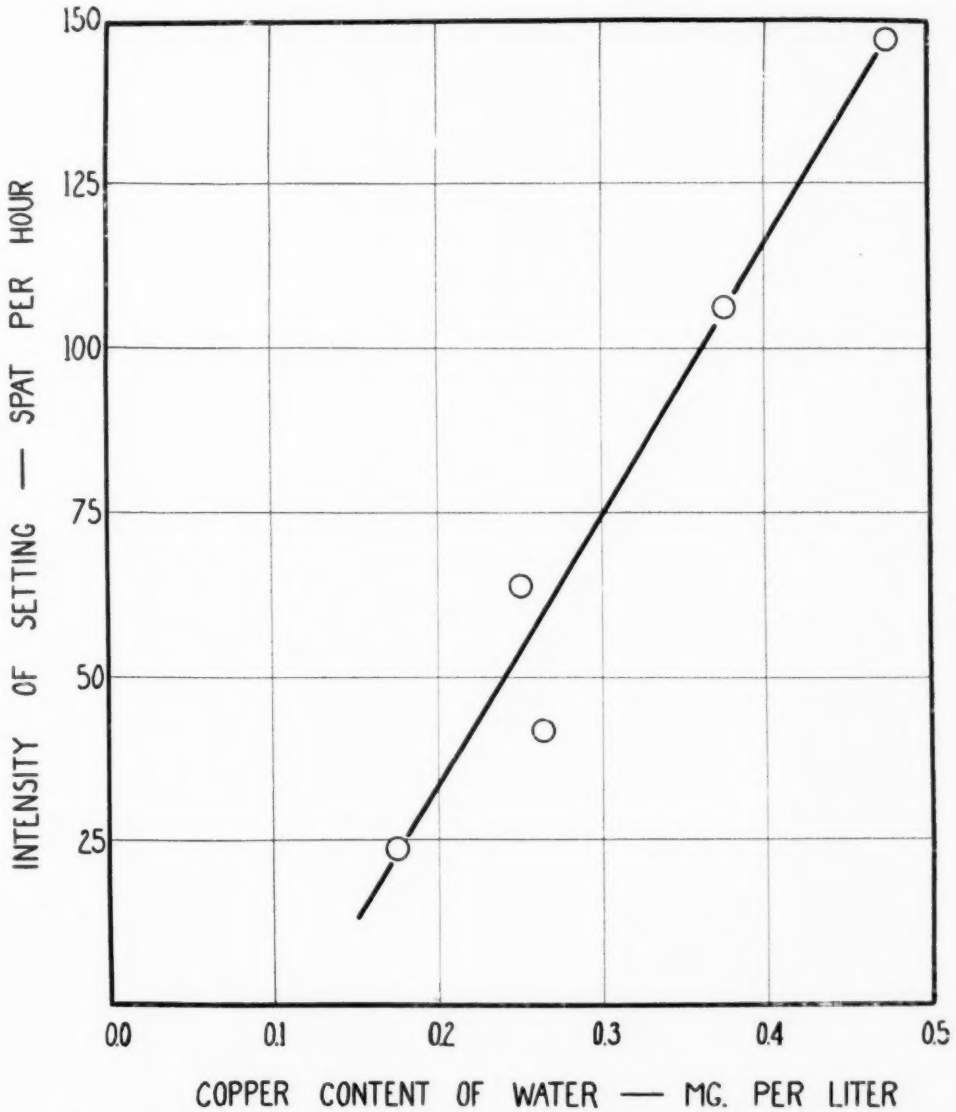

FIG. 14. Relation between copper content of the water and intensity of setting.

While the tide is ebbing, the water samples taken at this station consist largely of a mixture of the salt water from Long Island Sound and fresh water from the Wepawaug River, the proportion of the latter increasing gradually in amount as the tide falls. However, on the first of flood tide the samples contain a considerable portion of the water that is carried upstream from the Indian River, which also empties into the harbor near the entrance.

TABLE 8. Intensity of setting on July 22 in relation to the copper content of the water.

Tide	Hours after high water	Spat collected per hour	Per cent of total spat collection	Average cu. content per hr. mg. p.l.
High water.....	0–1	0	0.00	0.005
Ebb.....	1–2	0	0.00	0.007
do.....	2–3	0	0.00	0.012
do.....	3–4	2	0.53	0.082
do.....	4–5	12	3.16	0.175
Low water.....	5–6	64	16.84	0.250
do.....	6–7	105	27.63	0.375
Flood.....	7–8	148	38.94	0.475
do.....	8–9	43	11.32	0.265
do.....	9–10	4	1.05	0.019
do.....	10–11	2	0.53	0.008
High water.....	11–12	0	0.00	0.006

This river runs ebb into the harbor for an hour or two while the tide there is running flood. The bed of the Indian River is actually higher than low water level in the harbor and consequently this stream nearly empties itself before it is suddenly checked by the rapid rise of the tide two hours after low water. The last of the water from this river showed a copper content varying from 0.8 to 1.2 milligrams per liter. It is carried upstream to the sampling station during the first two hours of flood tide and is responsible for the sudden rise in the concentration of copper at such times. The effectiveness of this water in stimulating the setting of oyster larvae was evident from the fact that as it passed over the tidal flats there occurred over a period of about 10 days the setting of over 100 million larvae on 5 acres of this area. However, as the tide rose rapidly above the 2 foot mark, the flow from Indian River stopped as did also the setting of the larvae when they are subjected to water having a higher salinity and a very low copper content of less than 0.01 mg. per liter.

Here we undoubtedly have an explanation of the apparent correlation between current velocity and setting as stated by Prytherch (1929), pages 487 and 493. Current velocity was presented in this previous work as merely a physical factor that could be correlated to a certain extent with larval distribution and setting. It was observed that during low slack water, the larvae were most abundant and heaviest setting took place at the surface, in the zone where the velocity of the current was least or practically zero. This relationship held only at times of low water when larvae were abundant, and consequently it was apparent that changes in some other factor or factors paralleled those in current velocity and exerted a greater influence on swimming and setting of the larvae. As the present studies show, these factors are salinity and particularly the copper content of the water. When the tide in Milford Harbor rises above the 2 foot mark the current velocity and salinity increase rapidly while there is a sudden drop in the copper content of the

water that is followed by cessation of the swimming and setting activities of the larvae.

Studies of the relation between current velocity and distribution of larvae have also been made in New Jersey waters by Nelson and Perkins (1931) and differ in many respects from those reported by the author in 1929. The situation can be easily clarified, however, if one realizes that field observations in the two localities are not comparable as they differ markedly in respect to hydrographical and tidal conditions, amount of river discharge, relation of current velocity to stage of tide and particularly the changes in salinity that accompany variations in current velocity. Perkins reports an apparent correlation between the greatest abundance of larvae and highest current velocity. Since his observations on current velocity (expressed in revolutions rather than actual velocities) were made from August 16 to 25 and the larvae collections made from June 26 to August 3, such correlation is of questionable value. Practically all of his larvae were immature while those studied by the author were fully developed and of setting size. The failure of most larvae to develop pigment spots and reach setting size as reported by Perkins may be due, in the light of the present studies, to a deficiency of copper in these waters. The death of the heavy set of 1925 shortly after attachment as reported by Nelson in this same paper, page 22, may likewise be attributable to insufficient copper for metamorphosis as shown previously.

It is evident in the light of the present studies that the effects of current velocity on the larvae are secondary to those produced by chemical changes in the water. Increase in hydrogen-ion concentration, changes in salinity towards the optimum for setting (15 to 25 per mille) and traces of various elements such as copper, iron, manganese, etc., were observed by the author to stimulate greatly the swimming of the larvae. At certain stages of the tide such conditions may prevail and overshadow any effect of changes in current velocity. In setting the current is important as a physical agency that may be helpful in bringing the larvae in contact with submerged objects or detrimental when of such velocity as to prevent their attachment thereto.

The relation of current velocity to the distribution and setting of larvae in Milford Harbor or elsewhere should be considered chiefly in respect to the changes in chemical composition of the water that accompany this factor. Variations in copper content are particularly important and are within certain limits closely related to the intensity of setting as shown graphically in Figure 14. As the copper concentration increases from 0.175 to 0.475 mg. per liter, there is a corresponding increase in the intensity of setting ranging from 12 to 148 spat per hour per collector of the type shown in Figure 2. The setting varies directly as the copper content within these limits and shows that the mean rate of increase in intensity is 45.3 spat for each 0.1 milligram rise in copper concentration per liter of water.

These experiments demonstrate that under natural conditions copper is present in these inshore waters and is not only necessary for the setting of the oyster larvae, but must be present in sufficient concentration to initiate this reaction. During high water and intermediate tidal periods when the copper concentration was found to be less than 0.01 mg. per liter no evidence of setting could be found. With the ebbing of the tide the salinity gradually becomes lower and there is a steady increase in copper content as the inflow of river water into the harbor becomes greater. Consequently it was in the direction of the rivers and land that search was made for this important element in the environment of the oyster.

NATURAL SOURCE OF COPPER

The Wepawaug and Indians rivers are the largest fresh water streams flowing into Milford Harbor of which numerous analyses were made to determine to what extent copper was carried by them, down to the oyster beds. During the period from July 15, to September 15, 1930, the water from the Wepawaug River showed the presence of copper in amounts varying from 0.20 to 1.00 mg. per liter, with an average concentration of 0.65 milligram per liter. The Indian River for the same period showed a slightly higher copper content which varied from 0.45 to 1.60 milligrams per liter and averaged 1.20 mg. p.l. in samples taken 1 mile from its point of discharge into the harbor.

The coastal rivers should be considered as the primary agencies in the transportation of small amounts of this heavy metal from the land to the sea. The real source of the copper is the soil, rock materials, and mineral deposits which make up the land areas in each drainage basin. When they are subjected to the solvent action of the water from rain and snow, small amounts of copper and various elements may go into solution and thus be transported many miles to the sea. The route by which they are transported may be either overground or underground or a combination of the two. Rain water and river water are particularly active agencies in the chemical denudation of the land because they contain carbonic acid of either atmospheric or organic origin, which is the most abundant and generally the most potent of the agencies that dissolve mineral matter from the rocks.

In coastal regions the solvent action of rain and river water is further increased by their higher content of chlorides which are carried inland by winds and vapor from the ocean. In Connecticut, the normal chlorine content of surface waters varies from 6.7 parts per million on the coast to 1.2 parts per million at the northern boundary of the state as shown by Mason (1910) in the isochlor charts of New England. Copper is slightly soluble even in distilled water and goes into solution to an even greater extent in

natural waters containing carbonic acid and small amounts of various mineral salts.

Ores and rocks containing copper are widely distributed in nature and are quite plentiful in Connecticut where according to Hoffman (1924) the mining of copper was first carried on in the United States. Copper has also been mined in New Jersey, Pennsylvania, Virginia, North Carolina, and Georgia from the strata of massive pyrrhitous deposits that extend along the Atlantic coast from Newfoundland to Alabama. Appreciable amounts of this heavy metal are likewise found in nearly all soils, as shown from its absorption by plants in the stems, leaves, and seeds. McHargue (1927) found in forage crops, cereals, and plant food materials from 12 to 54 mg. of copper per kilogram while Elvehjem and Hart (1929) found from 2.2 to 89.5 mg. of copper per kilo (dry basis) in 47 common feeding stuffs for domestic animals.

As a stream travels in its downward course to the sea, we find that generally there is a gradual increase in its content of dissolved chemical substances and suspended solid matter, the amount of which will vary according to the physiographical features of each drainage basin. The transportation of these land materials ceases as the fresh water passes into the brackish water coastal areas at its mouth, where deposition of most of its load is brought about by the precipitating and coalescing action of the salt water. The rapid sedimentation of the suspended matter in tidal areas is shown remarkably well by Sale and Skinner (1917) and is significant in the present study as copper is likewise slowly precipitated by electrolytes in these same areas. This process is important because in the coastal regions where the mixing of fresh and salt water and the processes of precipitation and sedimentation are pronounced, we find the most prolific areas for the growth, reproduction, and setting of the oyster.

In analyses of river water little attention has unfortunately been paid to the presence of copper and the rarer elements, though it has been shown by Clark (1924) that the silt brought down by the Mississippi River contains 0.0043 per cent of cupric oxide (CuO). The quantity of silt delivered annually by the Mississippi to the Gulf of Mexico is estimated at approximately 370,000,000 metric tons of which 15,910 tons consist of this copper compound. At the mouth of the Mississippi, oysters are found in extreme abundance and setting is heavy which is due to a certain extent to the copper that is carried down and deposited by the river in this region.

Observations by the author in Virginia and North Carolina show that underground waters are likewise an important agency in the dissolution and transportation of copper to coastal areas. In the Seaside region of Virginia, where there are no rivers and but little surface drainage, the setting of oysters was found to be very heavy on many areas which were but a few

miles from the open ocean. The examination of this water showed, however, that its salinity, after the prolonged dry spell of 1930, was from 27.5 to 28.8 parts per thousand at the time of low water, which is approximately the same as that shown in Table V for Milford Harbor. This means that there must be an underground flow of fresh water into these areas in sufficient quantity to reduce the incoming ocean water from a salinity of 33. parts per thousand to the observed concentration. There is here an outflow of subterranean waters, through springs located both above and below tide marks, which is relatively equal to that of surface waters in other regions. Underground waters flowing into this region must likewise carry appreciable amounts of copper as the low water samples taken near Seaside, Va., showed that it was present in concentrations ranging from 0.08 to 0.25 mg. per liter.

An excellent example of the discharging of subterranean waters at sea level is found on the southern side of Long Island in the Brooklyn water supply system. Here nearly 40 per cent of the water from precipitation and snowfall in an area of 73 square miles passes into the earth and emerges from springs at the rate of over 40,000,000 gallons per day, a large amount of which is discharged along the shores of the bays and passes into the sea.

Thus we arrive at the conclusion, that (1) the land is the chief natural source of the copper that is found in the water of inshore coastal areas, (2) that through the mechanical and chemical action of water from rain and snow, this element is dissolved in small amounts from the land and conveyed to the sea by surface and underground waters, (3) as the fresh water from the land passes into the sea, the copper is rapidly precipitated and gradually assimilated to a large extent in the tissues of marine plants and animals, and (4) the continual denudation of the land by physical and chemical processes is of fundamental importance in the fertility of the coastal areas as it constitutes the chief supply of the organic and inorganic elements that are essential for the development and reproduction of marine organisms.

DISTRIBUTION OF OYSTERS

Oysters are distributed primarily in the littoral zone and shallow inshore areas along the coast where they are the most important and valuable marine form. In general their vertical distribution extends from nearly high water mark to a depth of 5 to 10 fathoms, while horizontally they are found in the coastal areas where the salinity of the water is intermediate between that of the ocean and river water.

The location of the natural oyster beds and shell deposits along the coast indicates that certain regions are more favorable than others for the growth and propagation of the oyster. The favorable regions for the most part lie in the coves, bays, and estuaries and are all similar in structure, consisting generally of a partially inclosed basin, which receives fresh water from the

land and salt or brackish water from the place into which it empties. Within these areas the productivity and abundance of oysters show wide variations in accordance with certain physical and chemical conditions.

The most prolific zones are those where the heaviest setting occurs which can now, according to the previous studies, be correlated quite definitely with the copper content and salinity of the water. In different regions these heavy setting zones may show marked differences in their vertical distribution, as shown in Figures 15 and 16. In these localities the range of tide is approximately the same, but the distribution of oysters in respect to low water mark is decidedly different. Studies by the author show that in each instance, the level at which heaviest setting occurs corresponds with the stage of tide when salinity is lowest and the content of copper and river water greatest. In one case, Figure 16, this level comes about half way between high and low water mark while in another, Figure 15, it extends from a foot below to a foot above low water level. The vertical difference in the location of these levels is due to different tidal conditions as a result of which, we have, in the first instance, slack water at half tide level and the lowest salinity and highest copper content (0.35 mg. p.l.) at the time of slack before flood. In the second case, in Connecticut, we have slack water at the time of high and low water but the lowest salinity and highest copper content occur only during the low water stage of the tide. It can be seen, therefore, that the distribution of oysters in these intertidal zones is determined primarily by the higher copper content of the water at these levels and its stimulating effect on setting of the larvae.

In other localities we find heavy setting areas at a depth of several fathoms below low water mark, as for example, in Long Island Sound on the area to the westward from the mouth of the Housatonic River. The movement of the tides and currents over this region have been studied and described by Prytherch (1929) and show that the water from the Housatonic River has been a most important factor in producing favorable conditions there for setting. The water flowing from this river on the last of ebb tide is carried westward by the flood tide in the sound, so that there is distributed in this direction a large volume of water of low salinity and high copper content. Immediately to the eastward from the Housatonic the setting of oysters is negligible though at one time they did exist here in abundance on what was formerly known as the "Pompey" natural bed, until the course of this stream was altered by government improvements.

In the deep water areas the larvae may receive copper stimulation while on the bottom through the precipitation and deposition of this element as a metacolloid of copper, or by rising to the surface for a short interval, and there ingesting a sufficient amount of this substance to induce setting. In

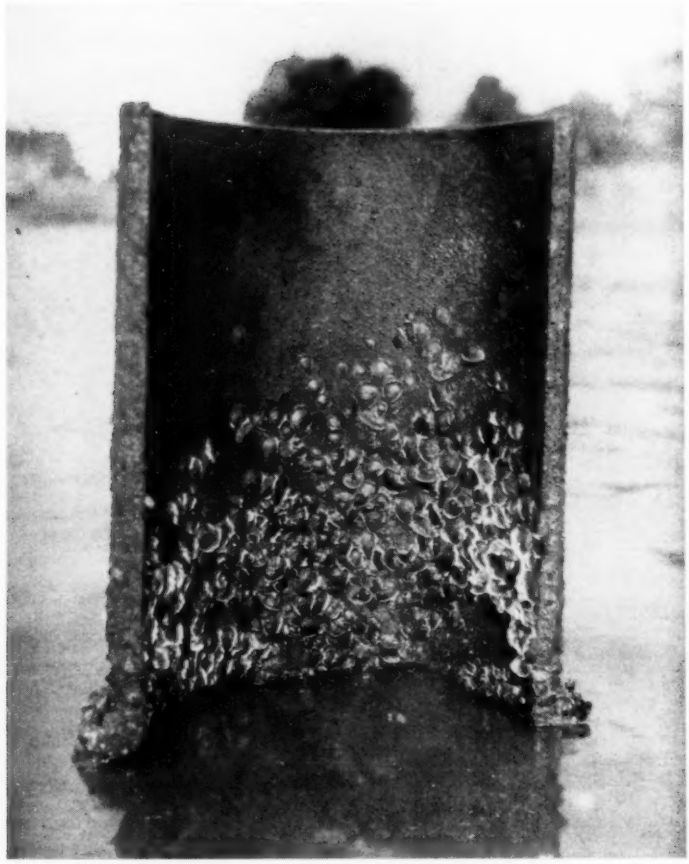

FIG. 15. Distribution of oysters above low water mark, Milford Harbor, 3 months old spat on glazed tile collector.

plankton samples the full grown larvae were collected occasionally at the surface over these areas, while earlier larval stages were rarely found.

There is also the possibility that submarine springs may flow into some of these deep water areas and supply copper in such amount as to produce more favorable conditions for setting, metamorphosis, and the production of seed oysters.

DISCUSSION

Researches of recent years have shown that copper is present in small amount in nearly every plant and animal. In man, measurable quantities have been found in the brain, blood, and liver, and it is believed that traces of this element are present in every other tissue as well. The significance of this element in human development is indicated by the studies of Bodansky (1921) and Morrison and Nash (1930). These authors found a much higher copper content in the human fetal brain than in the adult, and over 6 times as much copper in the livers of infants as in grown individuals. In one case

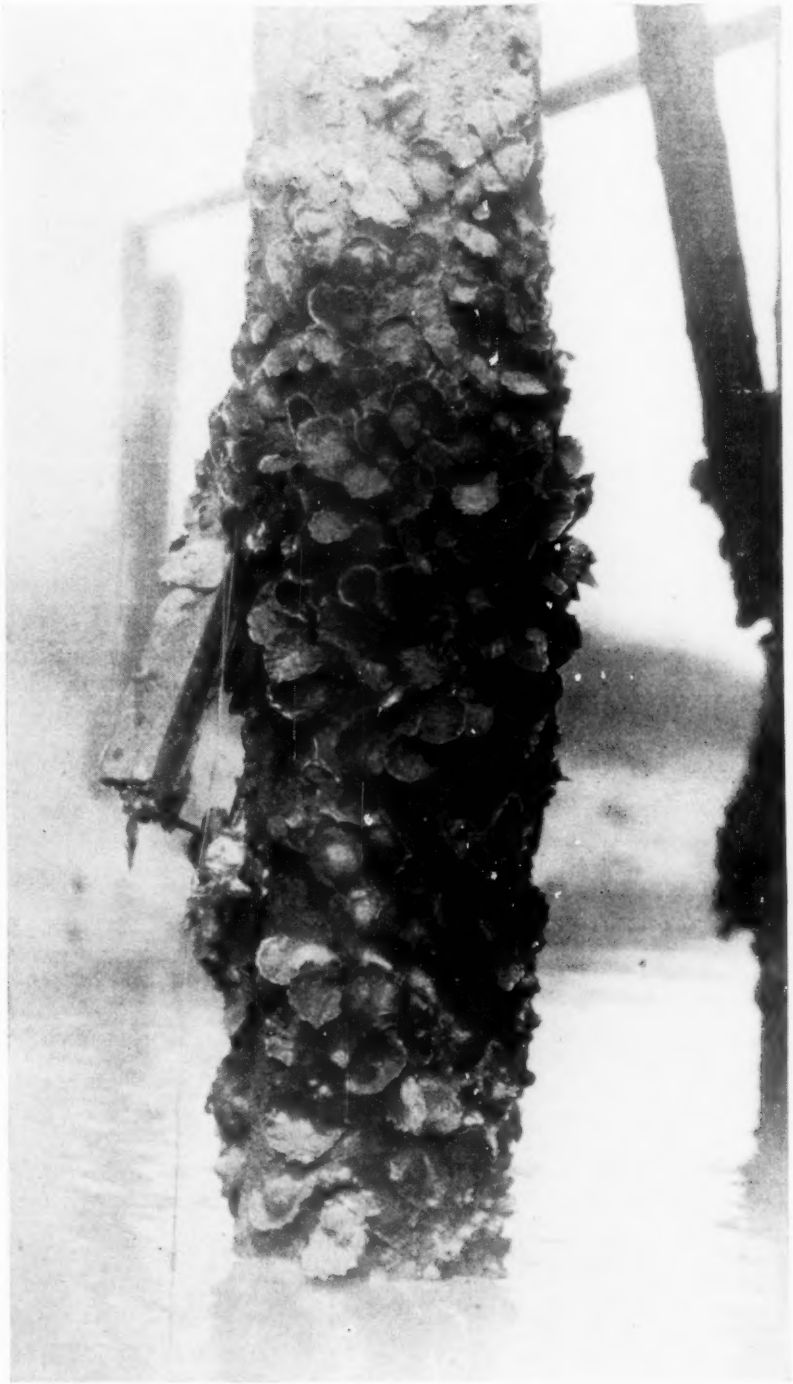

FIG. 16. Distribution of oysters above low water mark, Charleston Harbor, South Carolina. Oysters one year old on new piling.

where the copper content of the liver was but one-fourth the average amount, the death of the infant was due to severe anemia, which indicates a close relation between this element and hemoglobin formation.

In some of the lower forms, particularly marine species, this metal occurs in proportionally greater amount (Rose and Bodansky, 1920). Oysters contain more copper per unit of weight than any other animal which renders them particularly valuable in our diet. Recent studies by Levine, Remington, and Culp (1931) show that oysters contain considerable amounts of copper, iron, and manganese and that these elements are responsible for the anti-anemic potency of this seafood.

Previously various beneficial powers have been attributed to copper, such as the cure of nutritional anemia by Hart and his associates (1928) and Waddell (1929); the increased production of hemoglobin by McHargue, Healy, and Hill (1928) and Flinn and Inouye (1928); the increase of the oxygen carrying capacity of the blood by Flinn (1928); improvement in the fertility of the soil and plant growth by Bryan (1929); the increase of respiration in plants by LeVan (1930); etc. On the other hand, the toxic properties of this element in comparatively low concentrations have made it very valuable for the control or destruction of harmful plants and animals.

Glutathione is a substance of almost universal distribution in living cells, where it performs an important rôle in oxidation processes, and Meldrum and Dixon (1930); Elliott (1930), and others have shown that copper or iron are necessary for this function and have a marked catalytic effect upon it.

In mollusks and most crustacea copper is an essential constituent of the blood, where its combination with haemocyanin is analogous to that of iron in the hemoglobin of higher animals. The efficiency of the haemocyanin in the transport of oxygen in different marine animals is proportional to its copper content. Copper may thus be one of the significant factors in determining the muscular activity of different mollusks, and control to some extent their growth and general metabolism.

The rôle of copper in the development and distribution of the oyster serves to emphasize how important a single inorganic element may be in the ecology, physiology, and survival of an organism. A review of the life cycle and ontogeny of the oyster and particularly the critical periods of attachment and metamorphosis, when copper is such an essential and necessary element, leads us to believe that this heavy metal has also influenced greatly the evolution of this mollusk. In the adult oyster, copper is present in relatively large amount, and according to Taylor (1925) is rather generally distributed throughout the tissues, where it apparently performs an important function in oxidation processes. In the preceding stages when growth and differentiation is more rapid we find that copper must be present in the environment in low concentrations so as to supply this necessary element and stimulus for

development. In the setting and subsequent development of the oyster we have what appears to be a recapitulation of the most important period in the evolutionary history of this species. During this phase of its life cycle the oyster undergoes comparatively rapid modifications in its mode of existence as it changes from a free swimming to crawling and finally sedentary form. At the same time its internal and external organization likewise undergoes marked morphological changes. Since this series of events is induced specifically by copper it is possible that the presence of this element in its ancestral environment has had a decided influence on the evolution of the oyster and similar invertebrates. It seems likely in the light of the present studies made in the littoral zone or so called "cradle of evolution" that inorganic elements such as copper, iron, etc., may have served as important stimuli in inducing modifications in animal structure and behavior.

The life cycle of other allied species, such as *Mytilus edulis*, *Mya arenaria*, and *Teredo navalis*, parallels that of the oyster, and similar reactions of the larvae of these forms to copper were found in several instances. It is interesting to note that their distribution in Milford Harbor is identical with that of the oyster, and that their zone of heaviest setting is likewise just above and below low water level. The setting of these marine animals occurs when the salinity is lower and, in the case of the oyster, is at an optimum salt concentration, that is about half of that of the present ocean. This may be a further indication similar to that suggested by McCallum (1904 and 1930), that these comparatively rapid developmental changes occurred originally in the pre-Cambrian seas when the salinity of the water was lower.

Aside from theoretical considerations it is evident that the copper content of the water is a factor of considerable practical importance in the culture of the oyster. The present studies show that the setting of the oyster is a specific reaction which requires a definite chemical stimulus for its initiation. Copper is the only known chemical element capable of inducing this reaction and is effective in exceedingly small amounts. Copper was also found to be essential for the subsequent metamorphosis of the larva into a spat and adult oyster.

Under natural conditions copper is present in the water in minute concentrations. The oyster beds receive this element through chemical denudation of the land from which it is leached by surface and underground waters and transported to the sea. In the inshore coastal waters the copper content has been found to vary considerably in relation to tidal and hydrographical conditions and is largely responsible for the peculiar distribution of oysters in definite zones and their concentration at certain tidal levels. When copper is present in greatest amount the setting of the oyster larvae is most intensive. The number of larvae responding to stimulation by this element is directly proportional to the amount present. Consequently this is the primary en-

vironmental condition which determines the value of certain areas for setting and seed oyster production. It is also evident from these studies that trade wastes supplying copper will be highly toxic to the oyster larvae when the concentration of this metal is in excess of that found in natural waters. On the other hand, the valuable seed areas may also be seriously impaired if the natural supply of copper in river and underground waters is diminished by trade wastes of an alkaline character, or by the diversion of these waters to other drainage basins.

SUMMARY

1. The anatomy of the full grown oyster larva has been illustrated and described together with the functioning of the organs employed in feeding, swimming, setting, etc.

2. A complete description is given of the process by which the larva cements itself to submerged objects at the termination of its existence as a motile organism.

3. This process of attachment occurs under certain environmental conditions, the investigation of which has shown that the factors of salinity and river discharge are of primary importance.

4. The stimulus for setting has been traced to the surface and underground waters flowing into the inshore coastal areas, and specifically to the presence of minute amounts of copper in these waters.

5. The duration of the setting process varied in waters of different salinity and reached its optimum or shortest interval in salt concentrations of 16 to 18.6 per mille, when setting is completed in from 12 to 19 minutes.

6. Under natural conditions the setting of oyster larvae was most pronounced at the stage of tide when the copper content of the water was highest, and within a range of from 0.05 to 0.6 mg. of copper per liter.

7. Within this range the intensity of setting or number of individuals responding to copper stimulation was directly proportional to the amount of copper present.

8. The natural source of copper is the land in each drainage basin from which the metal is dissolved and transported by surface and underground waters to the oyster growing areas.

9. The oyster larva receives the stimulus for setting through the ingestion of the copper in the form of a colloidal precipitate and reacts to its presence after an average latent period of 4 minutes and 20 seconds.

10. Copper is highly toxic to the larva when the concentration is in excess of that found in natural waters, and gradually produces cytolysis of the tissues and death of the organism.

11. The metamorphosis of the attached larva and its development into a spat are dependent upon the ingestion of additional amounts of copper, which

initiates the breaking up of the pigment spots and the passage of approximately 600 pigmented cells into the blood stream.

12. The horizontal and vertical distribution of oysters in different coastal regions can be correlated with the copper content, salinity of the water, and the variations in these factors under different hydrographical and tidal conditions.

13. The location of the most prolific areas for setting and seed oyster production is determined largely by the degree of interaction between fresh water and sea water and particularly by the amount of copper supplied by the former.

BIBLIOGRAPHY

Anson, M. L., and A. E. Mirsky. 1930. Hemoglobin, the heme pigments and cellular respiration. Physiol. Rev., **10:** 506-546.

Bodansky, Meyer. 1921. The zinc and copper content of the human brain. Biol. Chem., **48:** 361-364.

Brenchley, Winifred E. 1927. Inorganic plant poisons and stimulants. Cambridge Agric. Monog. London.

Bryan, O. C. 1929. Stimulating effect of external applications of copper and manganese on certain chlorotic plants of the Florida Everglades soils. Jour. Soc. Agron., **21:** 923-933.

Callan, Thomas and Henderson, and J. A. Russell. 1929. A new reagent for the colorimetric determination of minute amounts of copper. Analyst, **54:** 650-653.

Clarke, Frank W. 1924. The data of geochemistry. Bull. 770, U. S. Geol. Sur. Washington.

Cook, S. F. 1926. The effect of certain heavy metals on respiration. Jour. Gen. Physiol., **9:** 575-601.

1926a. Latent period in the action of copper on respiration. Jour. Gen. Physiol., **9:** 631-650.

Elliott, Kenneth Allen Caldwell. 1930. On the catalysis of the oxidation of cysteine and thioglycollic acid by iron and copper. Biochem. Jour., **24:** 310-326.

Elvehjem, C. A., and E. B. Hart. 1929. The copper content of feeding stuffs. Jour. Biol. Chem., **82:** 473-477.

Elvehjem, C. A., and C. W. Lindow. 1929. The determination of copper in biological materials. Jour. Biol. Chem., **81:** 435-443.

Elvehjem, C. A., H. Steenbock, and E. B. Hart. 1929. Is copper a constituent of the hemoglobin molecule? The distribution of copper in blood. Jour. Biol. Chem., **83:** 21-25.

Elvehjem, Conrad Arnold. 1930. Factors affecting the catalytic action of copper in the oxidation of cysteine. Biochem. Jour., **24:** 415-426.

Field, Irving A. 1922. Biology and economic value of the sea mussel, *Mytilus edulis*. U. S. Bur. Fish., Bull., **38:** 127-259.

Flinn, Frederick B., and J. M. Inouye. 1928. Metals in our food. Jour. Amer. Med. Assoc., **90:** 1010.

1929. Some physiological aspects of copper in the organism. Jour. Biol. Chem., **84:** 101-114.

Galtsoff, Paul S., and Dorothy V. Whipple. 1930. Oxygen consumption of normal and green oysters. U. S. Bur. Fish., Doc. 1094, Bull., **46:** 489-508.

Galtsoff, P. S., H. F. Prytherch, and H. C. McMillin. 1930. An experimental study in production and collection of seed oysters. U. S. Bur. Fish., Doc. 1088, Bull., **46:** 197-263.

Hart, E. B., H. Steenbock, J. Waddell, and C. A. Elvehjem. 1928. Iron in nutrition. VII. Copper as a supplement to iron for hemoglobin building in the rat. Jour. Biol. Chem., **77:** 797-812.

Hofman, H. O. 1924. Metallurgy of copper. New York and London.

Hopkins, A. E. 1931. Factors influencing the spawning and setting of oysters in Galveston Bay, Texas. U. S. Bur. Fish., Bull., **47:** 57-83.

Huxley, J. S. 1922. Dedifferentiation in Echinus larvae and its relation to metamorphosis. Biol. Bull., **43:** 210-237.

Koehring, Vera. 1930. The neutral red reaction. Jour. Morph. and Physiol., **49:** 45-137.

Levan, William Christian. 1930. The effect of metals on the respiration of *Lupinus albus*. Amer. Jour. Bot., **17:** 381-395.

Levine, Harold, Roe E. Remington, and F. Bartow Culp. 1931. The value of the oyster in nutritional anemia. Jour. Nutrition, **4:** 469-481.

Lindow, C. W., C. A. Elvehjem, and W. H. Peterson. 1929. The copper content of plant and animal foods. Jour. Biol. Chem., **82:** 465-471.

Lindow, C. W., W. H. Peterson, and H. Steenbock. 1929. The copper metabolism of the rat. Jour. Biol. Chem., **84:** 419-436.

Macallum, A. B. 1904. The palaeochemistry of the ocean in relation to animal and vegetable protoplasm. Trans., Canadian Inst., Canada.

1930. Evolution traced biochemically. In Human biology and racial welfare by E. V. Cowdry. New York.

Marmer, H. A. 1926. The tide. New York and London.

Mason, William P. 1910. Examination of water (chemical and bacteriological). New York.

Mathews, A. P. 1905. The nature of chemical and electrical stimulation. The tension coefficient of salts and the precipitation of colloids by electrolytes. Amer. Jour. Physiol., **14:** 203-230.

McHargue, J. S. 1927. The proportion and significance of copper, iron, manganese, and zinc in some mollusks and crustaceans. Kentucky Acad. Sci., **2:** 1-7.

1927a. The significance of small amounts of inorganic elements in plants. Proceed. First International Congress of Soil Science, **3:** 1-7.

McHargue, J. S., D. J. Healy, and E. S. Hill. 1928. Relation of copper to haemoglobin content of rat blood. Jour. Biol. Chem., **78:** 637-641.

Meldrum, Norman Urquhart, and Malcolm Dixon. 1930. The properties of pure glutathione. Biochem. Jour., **24:** 472-496.

Morrison, Dempsie B., and Thomas P. Nash, Jr. 1930. The copper content of infant livers. Jour. Biol. Chem., **88:** 479-483.

Muttkowski, Richard A. 1920. Copper. Its occurrence and rôle in insects and other animals. Anat. Rec., **20:** 230. Philadelphia.

Nelson, Thurlow C. 1924. The attachment of oyster larvae. Biol. Bull., **46:** 143-151.

1924-1930. Report, Department of Biology. New Jersey Agric. Coll. Exp. Sta.

Nelson, Thurlow C., and Earle B. Perkins. 1931. Annual Report, Department of Biology. New Jersey Agric. Exp. Sta.

Prytherch, Herbert F. 1929. Investigation of the physical conditions controlling spawning of oysters and the occurrence, distribution, and setting of oyster larvae in Milford Harbor, Conn. U. S. Bur. Fish., Bull., **44:** 429-503.

1930. Improved methods for the collection of seed oysters. U. S. Bur. Fish., Doc. **1076:** 47-59.

191. The rôle of copper in the setting and metamorphosis of the oyster. Science, n. s., **73:** 429-431.

Rose, Mary S. 1929. What place have aluminum, copper, manganese, and zinc in normal nutrition. Jour. Nutrition, **1:** 541-556.

Rose, William C., and Meyer Bodansky. 1920. Biochemical studies on marine organisms 1. The occurrence of copper. Jour. Biol. Chem., **44:** 99-111.

Sale, J. W., and W. W. Skinner. 1917. The vertical distribution of dissolved oxygen and the precipitation by salt water in certain tidal areas. Jour., Franklin Inst., 837-848.

Stafford, J. 1909. On the recognition of bivalve larvae in plankton collections. Contrib. Canadian Biol., Report **14:** 221-242.

1913. The Canadian oyster. Its development, environment, and culture. Commission of Conservation, Canada. Ottawa.

Taylor, Harden F. 1926. Mineral constituents of fish and shellfish. In Nutritive value of fish and shellfish. Appendix 10. Report U. S. Commissioner of Fisheries for 1925. 539-546.

True, Rodney H. 1930. The toxicity of molecules and ions. Proc. Amer. Phil. Soc., **69:** 231-245.

Truitt, R. V. 1920-1925. Annual report of Maryland Conservation Department.

Voegtling, Carl, J. M. Johnson, and Helen A. Dyer. 1925. Protoplasmic action of copper and gold. Proc., Nat. Acad. Sci., **11:** 344-345.

Waddell, J., H. Steenbock, and E. B. Hart. 1929. Iron in nutrition. X. The specificity of copper as a supplement to iron in the cure of nutritional anemia. Jour. Biol. Chem., **84:** 115-130.

Warburg, O., and H. A. Krebs. 1927. Loosely combined copper and iron in blood serum. Biochem. Zeit., **190:** 143-149.

Washington, H. S. 1916. An apparent correspondence between the chemistry of igneous magmas and of organic metabolism. Proc., Nat. Acad. Sci., **2:** 623-626.

1920. The chemistry of the earth's crust. Jour., Franklin Inst., **190:** 757-815.

Willard, J. T. 1908. On the occurrence of copper in oysters. Jour. Amer. Chem. Soc., **30:** 902-904.

Yonge, C. M 1926. Structure and physiology of the organs of feeding and digestion in *Ostrea edulis*. Jour. Mar. Biol. Assoc., n. s., **14:** 295-386.

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